REMARKS

I. Claim Status. Claims 1-5 and 19-21 are pending.

(i) Claim Amendments: Claims 1 and 2 have been amended. Claim 1 has been amended to depict member B as being directly attached to the piperazine ring as originally described in the specification (page 3, line 2). Claim 1 has also been amended to remove C-$CONH_2$ as a member of the Markush group from which Y may be selected. The removal a Markush group member from claim 1 does not add new matter to the claim.

Claim 1 has been further amended, to recite that member B may be selected from the group consisting of (i) phenyl, (ii) naphthyl, (iii) benzodioxanyl, and (iv) indolyl; wherein a phenyl ring of said B is optionally substituted. Support for the amendment is found in the specification at page 3, lines 23, page 5, lines 17-25, Example 2, page 15 and Example 16, page 24. The proviso of claim 1 has also been amended to remove the C-$CONH_2$ as a member from which Y may be selected and to define member B. Support for the amendment is found in the specification at page 3, lines 20-27.

Claim 2 has been amended to remove C-$CONH_2$ as a potential member for the Y variable, in order to be consistent with claim 1. The removal of the Markush group member does not add new matter. Claim 2, has also been amended, correcting a typographical error, to recite the member CN as being C-CN. Accordingly, the amendment of Claim 2, does not introduce new matter into the specification.

(ii) Allowed Claims: Claims 3 and 19-21 have been allowed.

II. Claim Rejections. The rejections of the claims are summarized and addressed as follows:

(i) Rejection under 35 U.S.C. § 112, second paragraph.

The Examiner has rejected claims 1, 2, 4 and 5 as being allegedly indefinite. The Examiner asserts that the specification only recites the bicyclic structures; naphthyl, benzodioxanyl, and indolyl and objects to the preamble to the definition of substituent B, subpart (b) in claim 1, which allegedly broadens the claim to include a multitude of bicyclic structures. The Examiner also objects to the term "CN" in claim 2.

In response, without conceding the correctness of the rejection, claim 1 has been amended. Claim 1 now recites that member B may be selected from the group consisting of (i) phenyl, (ii) naphthyl, (iii) benzodioxanyl, and (iv) indolyl; wherein a phenyl ring of said B is optionally substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, cyano, nitro, amino, alkylsulfonylamino and alkylamino. The applicants submit that amended claim 1, points out and distinctly claims the subject matter of the present invention. The specification clearly discloses that B may be a substituted phenyl (page 3, lines 17-22), naphthyl (page 3, lines 18-19), benzodixanyl (Example 2, page 15), or indolyl (Example 16, page 24).

Claim 2 has been amended to recite C-CN rather then CN, as suggested by the Examiner. The amendment was made to correct a typographical error.

Applicants respectfully submit that amendments to claims 1 and 2 address all the rejections of the claims under 35 U.S.C. § 112, second paragraph. Accordingly, applicants respectfully request reconsideration of claims 1, 2, 4 and 5 and withdrawal of all rejections thereof under 35 U.S.C. § 112, second paragraph.

(ii) Rejections under 35 U.S.C. § 112, first paragraph

(a) *Written Description*. The Examiner has rejected claims 1, 2, 4 and 5 for containing subject matter which is not allegedly described in the specification in such a way as to

reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed.

In response, without conceding the correctness of the Examiner's position, claim 1 has been amended to depict member B directly attached to the piperazine ring and to specify that B is either a phenyl ring, naphtyl, benzodixanyl or indolyl, where the phenyl ring of member B is optionally substituted. Support this amendment is found in the specification at page 3, lines 23, page 5, lines 17-25, Example 2, page 15 and Example 16, page 24. Proviso (1) of claim 1, has also been amended to recite that Ar and Ar' cannot be simultaneously unsubstituted when Y is C-N and B is methoxyphenyl. The support for the amended proviso is found in the specification at, page 3, lines 26-27 and Example 15, page 23.

The amendment of claim 1 adopts amendments suggested by the Examiner. Hence, the present rejection of claim 1 is believed to have been addressed and overcome.

(b) Enablement. The Examiner has also rejected claims 1, 2, 4 and 5 for alledgedly lacking of support in the specification sufficient to enable one skilled in the art to practice the claimed invention.

In response, without conceding the correctness of the Examiner's position, the proviso of claim 1 has been amended to recite that Ar and Ar' cannot be simultaneously unsubstituted when Y is C-N and B is methoxyphenyl. Support for the amended proviso is found in the specification at page 3, lines 26-27 and Example 15, page 23. Applicants submit that based upon the description and examples of the specification, one skilled in the art would be able to practice the full scope of the invention of claim 1. The present rejection is therefore believed to have been addressed and overcome.

For all the reasons set forth above, Applicants believe that all of the rejections of claims 1, 2, 4 and 5 under 35 U.S.C. § 112, first paragraph have been addressed and overcome.

Applicants respectfully request reconsideration of claims 1, 2, 4 and 5 and withdrawal of all rejections thereof under 35 U.S.C. § 112, first paragraph, accordingly.

(iii) Rejection under 35 U.S.C. § 103(a)

The Examiner has again rejected claims 1, 2, 4 and 5, as obvious over Janssen (US. Patent No. 3,030,367) and Shiota (WO 97/44329).

The Examiner again, has specifically cited Shiota for compounds in Table 1.1, nos. 1 and 7-9. With respect to Shiota compound 7, Applicants submit that it is not structurally similar to any of the claimed compounds. Using the nomenclature of the present claims, in Shiota's compound 7, "B" is methylsulfonyl, which differs from the claims wherein "B" is " alkylsulfonylamino, by the absence of the amino group. Shiota fails to suggest that compound 7 be modified to include the amino group. Hence, the instant claims are not obvious over compound 7.

With respect to the remaining compounds of Shiota, applicants submit a verified translation of Italian Application No. MI97A 001861, filed August 1, 1997, from which the present application claims priority under 35 U.S.C.§ 119(a)-(d), together with a statement that the translation ~~is a true and complete translation of said Italian application. The Italian priority document was filed~~ before the November 27, 1997 publication date of Shiota.

The Italian priority document discloses compounds wherein, Ar and Ar', separately represent a substituted or non-substituted aryl group, the term Y represents a CH or C-CN group, R represents a hydrogen atom, Z represents a methylene group, Z' represents a valence bond and B represents an unsubstituted aryl group or an aryl group substituted with one or more halogen atoms or alkyl or cyano groups (see translation at page 1, final paragraph and page 2, first paragraph of "DETAILED DESCRIPTION OF THE INVENTION.") Hence, Shiota is not

available as prior art against the instant claims. Reconsideration of claims 1, 2, 4 and 5 and withdrawal of rejection under 35 U.S.C. § 103 thereof based on Shiota are therefore requested.

Claims 1, 2, 4 and 5 remain rejected as obvious over Janssen. In response, without conceding the correctness of the rejection, claim 1 has been amended to recite that Y may not be C-$CONH_2$, and to recite in the proviso that when Y is C-CN, and B is methoxyphenyl, then Ar and Ar' may not be simultaneously unsubstituted. Accordingly, the present amendment claims compounds distinct from those disclosed in Janssen. The compounds of Janssen, particularly the compound of example 4, are distinguished from the compounds of claim 1, as the Janssen compound depicts Y being C-$CONH_2$. Furthermore, Janssen does not include any suggestion or motivation to modify the compounds disclosed therein to arrive at the presently claimed compounds. Accordingly, claims 1, 2, 4 and 5 are not obvious over Janssen. Applicants respectfully request reconsideration of claims 1, 2, 4 and 5 and withdrawal of all rejections thereof under 35 U.S.C. §103, accordingly.

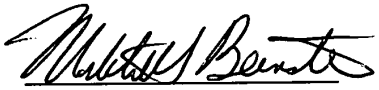
CONCLUSION

This application is believed to be in condition for allowance, which is earnestly solicited.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,

Dated: November 12, 2003



Mitchell Bernstein, Ph.D.
Reg. No. 46,550
Agent for Applicant(s)

DARBY & DARBY P.C.
Post Office Box 5257
New York, NY 10150-5257
212-527-7700

EXPRESS MAIL CERTIFICATE

Date 11-12-03 Label No. EV340064339US

I hereby certify that, on the date indicated above, this paper or fee was deposited with the U.S. Postal Service & that it was addressed for delivery to the Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450 by "Express Mail Post Office to Addressee" service.

PATRICIA A. RUBIO
Name (Print)

Patricia A. Rubio
Signature

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Docket No.: 6485/1D340-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Amedeo LEONARDI et al.

Serial No.: 09/127,059

Art Unit: 1624

Filed: July 31, 1998

Examiner: Emily B. Bernhardt

For: **DIARYLALKYLPIPERAZINES ACTIVE ON THE LOWER URINARY TRACT**

VERIFICATION OF A TRANSLATION

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the below named individual, hereby declare as follows:

My name and post office address are as stated below;

I am knowledgeable in the English language and in the language of the attached foreign language document, Italian Patent Application No. MI 97 A 001861, and I believe the attached English translation of that document is a true and complete translation thereof;

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified patent application or any patent issued thereof.

Dated: July 29, 2003

Translator's name (PRINTED) Mr Fabrizio CASTAGNO

Translator's Signature _____

Post Office Address

Recordati Industria Chimica e Farmaceutica S.p.A.

Via Matteo Civitali, 1 Milano 20148, ITALY

NOTE: COPY OF FOREIGN LANGUAGE DOCUMENT AND ENGLISH TRANSLATION MUST BE ATTACHED.

Form C.E. – 1-4-7

MINISTRY OF INDUSTRY, TRADE AND CRAFTSMANSHIP

GENERAL MANAGEMENT FOR INDUSTRIAL PRODUCTION

ITALIAN OFFICE FOR PATENTS AND TRADE MARKS

Certification of copies of documents regarding the patent application for an IND(ustrial) INV(ention) No. MI97 A 001861.

We certify that the enclosed copy is true to the original documents filed with the above patent application, details of which are shown in the enclosed filing report.

29 July 1998

DIVISION MANAGER

Ing. Attilio Roncacci

TO THE MINISTRY OF INDUSTRY, TRADE AND CRAFTSMANSHIP FORM A
CENTRAL PATENT OFFICE - ROME
PATENT APPLICATION FOR AN INDUSTRIAL INVENTION, FILING OF RESERVES, EARLY AVAILABILITY TO PUBLIC

A. APPLICANT (1) N.G.
1) Name **RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A.** **SP**
Address **MILAN - ITALY** code **00748210150**

C. Receiver's ELECTIVE ADDRESS RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A.
street **VIA MATTEO CIVITALI** No. **01** city **MILAN** zip code **20148** (prov) **MI**

D. TITLE proposed class (sect/cl/scl) **A61K**
DIARYLALKYLPIPERAZINES ACTIVE ON THE LOWER URINARY TRACT

EARLY AVAILABILITY TO PUBLIC : YES NO **X**

E. DESIGNATED INVENTORS
Surname and Christian name
1) **LEONARDI Amedeo**
2) **MOTTA Gianni**
Surname and Christian name
3) **RIVA Carlo**
4) **TESTA Rodolfo**

ENCLOSED DOCUMENTS

	No. of copies		
Doc. 1)	**1**	No. of pages **48**	summary with main drawing, description and claims (1 copy compulsory)
Doc. 2)		No. of figures	drawings (1 copy compulsory when referred to in description)
Doc. 4)	**1**		inventor designation

8) receipts of payment, total Lit. 565,000. - five hundred and sixty five thousand compulsory

COMPLETED ON **01/08/1997** SIGNATURE OF APPLICANT **Abraham SARTANI, R&D Manager**
RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A.

~~CONTINUED YES/NO NO~~

A CERTIFIED COPY OF THIS DEED IS REQUIRED YES/NO **YES**

C.C.I.A.A. OF **MILAN** CODE 15

FILING REPORT APPLICATION NUMBER MI97A001861 Reg. A
This FIRST day of the month of AUGUST of the year one thousand nine hundred NINETY SEVEN the above applicant submitted this application with No. 00 added sheets to the underwriter for granting the above patent.

THE APPLICANT Stamp THE DRAFTING OFFICIAL
M. CORTONESI

FORM A

SUMMARY OF INVENTION WITH MAIN DRAWING, DESCRIPTION AND CLAIMS

D. TITLE

"DIARYLALKYLPIPERAZINES ACTIVE ON THE LOWER URINARY TRACT"

L. SUMMARY

Described are diarylalkylpiperazine derivatives active on the lower urinary tract. These compounds and their enantiomers, diastereoisomers, N-oxides, polymorphs, solvates and pharmaceutically acceptable salts are useful in the treatment of patients afflicted with neuromuscular dysfunction of the lower urinary tract and diseases related to 5-HT_{1A} receptor. Also described are the preparation of the compounds and the pharmaceutical compositions containing them.

RECORDATI
Industria Chimica e Farmaceutica S.p.A.

M. DRAWING

[redacted]

Description of proposed invention by title:

"DIARYLALKYLPIPERAZINES ACTIVE ON THE URINARY TRACT"

By name: Recordati, Industria Chimica e Farmaceutica S.p.A.

Based in: Milan, via Civitali, 1 ;

Nationality: Italian.

Designated Inventors: Amedeo LEONARDI, Gianni MOTTA, Carlo RIVA and Rodolfo TESTA.

****

GOAL OF INVENTION

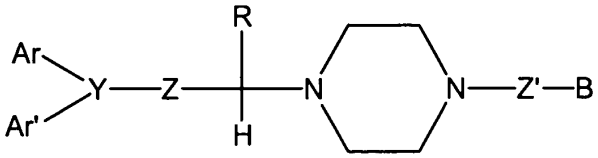
The present invention concerns diarylalkylpiperazine, the pharmaceutical compositions which contain it, and the use of such derivatives and compositions.

Flavoxate, imipramine and oxybutynin are the principal active representatives of the various classes of compounds which are currently employed in the therapeutic treatment of urinary incontinence. The activity of these compounds in the are in question has been verified through experimental studies with animal models.

The compounds which are the subject of the present invention, and which are defined and discussed in detail below, share few structural characteristics with flavoxate, imipramine and oxybutynin, with the exception of the presence of a basic nitrogen atom within the molecule.

The invention compounds have demonstrated a significantly higher level of activity than flavoxate, imipramine and oxybutynin, in pharmacological evaluations of effects produced upon the lower urinary tract, and, in particular, in regard to effective activity against neuromuscular dysfunctions of the lower urinary tract. The invention compounds exhibit a high degree of affinity for type 5-HT1A serotoninergic receptors.

In one of its forms, the present invention concerns compounds of the general structural formula I:



(I)

in which

the terms Ar and Ar' separately represent a substituted or non-substituted aryl group, or a substituted or non-substituted heteroaryl group,

Y represents a nitrogen atom or a CH, C-OH, C-CN or C-$CONH_2$ group,

R represents a hydrogen atom or a lower alkyl group,

B represents a substituted or non-substituted aryl group or a substituted or non-substituted heteroaryl group,

Z represents a methylene or ethylene group, and

Z' represents a valence bond or a methylene or ethylene group.

The present invention also comprises the enantiomers, diastereoisomers, *N*-oxides, polymorphs, solvates and pharmaceutically acceptable salts of such compounds.

In addition, the present invention includes pharmaceutical compositions which contain compounds of the general structural formula I, or their enantiomers, diastereoisomers, *N*-oxides, polymorphs, solvates and pharmaceutically acceptable salts, in mixture with pharmaceutically acceptable diluents and vehicles.

~~The present invention also involves the use of such compounds in the treatment of~~ patients afflicted with neuromuscular dysfunctions of the lower urinary tract, in particular, by means of a generated reduction in the frequency of bladder contractions of the type caused by bladder distension, as well as by the increasing of bladder capacity. The treatment regimen in question here involves the administration to such patients of a therapeutically effective quantity of one or more selected compounds of structural formula I, or the enantiomers, diastereoisomers, *N*-oxides, polymorphs, solvates or pharmaceutically acceptable salts of such compounds.

Another aspect of the present invention comprises the interaction of the invention compounds with type 5-HT1A serotoninergic receptors, an interaction which makes possible

the use of the invention compounds in the treatment of disturbances of the central nervous system, such as, for example, anxiety and depression, hypertension, disturbances in the sleeping/waking cycle, alimentary activity and/or sexual function, and cognitive disturbances, in mammals, and particularly in humans.

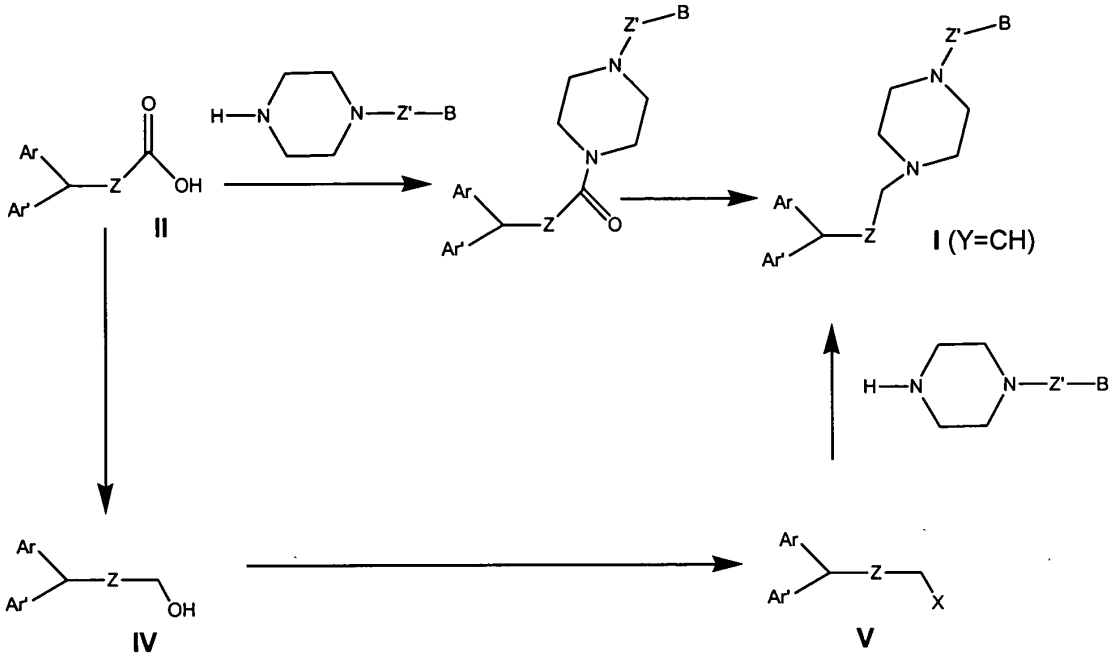
DETAILED DESCRIPTION OF INVENTION

The aryl groups which are represented in structural formula I under the terms B, Ar' and Ar' consist, by preference, of monocyclic or bicyclic groups which possess from 6 to 12 carbon atoms (such as, for example, phenyl or naphthyl). The heteroaryl groups which are represented in structural formula I under the terms B, Ar' and Ar' consist, by preference, of monocyclic or bicyclic groups which posses from 5 to 12 carbon atoms, one or more of which are heteroatoms (such as, for example, nitrogen, oxygen or sulfur), with the remainder being carbon atoms. One or more substituents may be selected from the following: halogen atoms and alkyl, alkoxy, halogalkoxy, cyano, carbamoyl, acyl, nitro, amino, acylamino, alkylsulfonylamino and alkylamino groups. When B represents an aryl group, two substituents of the aromatic ring may be joined together to form an additional ring. For example, B can represent a benzodioxanyl ring.

Synthesis of Compounds of the Invention

Compounds of the general structural formula I, which are the subject of the present invention, where Y is a CH, R is H and Ar, Ar', B, Z and Z' are defined as above, are normally prepared as indicated below in synthesis scheme 1:

Scheme 1



The initial compounds of formula II are either readily available commercially, or else their synthesis has been described in detail in the literature and/or they may be obtained through the use of traditional synthesis procedures. For example, from initial substances in which Z represents methylene, the corresponding diaryl ketones are obtained through the use of the Reformatsky reaction, with the use of alkyl 2-bromoacetate and activated zinc (*Org. React.*, 22 **1975**, 423; *Synthesis*, **1989**, 571). The obtained reaction product is then subjected to hydrolysis. In an alternative procedure, a Wadsworth-Emmons reaction is carried out initially, with the use of triethyl phosphonoacetate and base *(Chem. Rev.*, **1989**, 89, 863), which is followed by hydrolysis. Other well-known synthsis procedures may be employed by experienced practitioners.

The obtained initial type II intermediates may then be subjected to condensation with appropriate N-monosubstituted piperazine derivatives, in the presence of a suitable condensing agent (such as, for example, diethyl cyanophosphonate, dicyclohexylcarbodiimide or N,N-carbonyldiimidazole), and, possibly, in the presence of a suitable promoting agent (such as, for example, N-hydroxysuccinimide, 4-dimethylaminopyridine), in an aprotic or chloridated solvent (for example, N,N-dimethylformamide, chloroform, methylene chloride) at -20°C/140°C (Albertson, *Org.*

React., **1962**, 12, 205-218; Doherty et al., *J. Med. Chem.*, **1992**, 35, 2; Staab et al., *Newer Methods Prep. Org. Chem.*, **1968**, 5, 61; Ishihara, *Chem. Pharm. Bull.*, **1991**, 39, 3236).

Other available types of reaction include the mixed anhydriding method, which involves the reacting of the initial type II intermediate with a chloroformated alkyl, in the presence of a tertiary amine (for example, triethylamine), followed by the addition of the piperazine reagent within an aprotic solvent (for example, dioxane or methylene chloride), and, possibly, in the presence of a suitable promoting agent, such as 1-hydroxypiperidine (*Org. React.* **1962** 12,157).

Other potential methods for use in the amidification of the initial type II intermediate (or simple derivatives of type II compounds, such as esters or acyl chlorides), with *N*-monosubstituted piperazines, are well-known to those experienced in the art. Another possible condensation procedure involves the reacting of simple alkyl esters of type II intermediate compounds with an aluminum amide, which was previously obtained by means of the reacting of piperazine with trimethylaluminum (*J. Med. Chem.*, **1996**, 39, 4692).

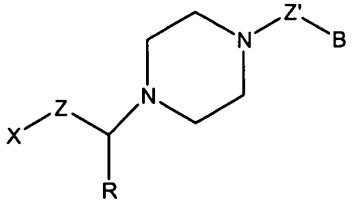
The obtained type III intermediate compounds may be reduced to the desired type (I) product compounds, where Y=CH, through the use of reducing agents, such as, for instance, lithium aluminum hydride, or other suitable hydride complexes, which are capable of converting the amide function into an amine function. Reactions of this type are carried out with diethyl ether or tetrahydrofuran, or, alternatively, in a stable diborane complex such as borane-tetrahydrofuran, borane-dimethyl sulfide, etc. (*J. Org. Chem.*, **1982**, 47, 1389), in a suitable solvent (for example, tetrahydrofuran). Boron compounds of this type are particularly effective in the role in question here, when the Ar groups bear reducible groups, such as nitro groups, which have not yet undergone reduction.

Other reducing agents which are suitable for use in this context are well known to those of skill in the art (March, *Advanced Organic Chemistry*, Wiley Interscience Ed., **1992**, 1212).

An alternative reaction pathway for use in the production of type (I) product where Y is CH, involves the reduction of type II compounds with the reducing agents described above, or through the use of other conventional techniques and materials (for example, $NaBH_4$ with $CaCl_2$, or reduction of the mixed anhydrides obtained in a reaction with a

chloroformate, with $NaBH_4$) in which alcohol compounds IV have been converted to alkylation reagents V, where X represents a leaving group (Cl, Br, I, p-toluenesulfonyloxy, methanesulfonyloxy), through the use of conventional techniques which are well-documented in the literature. The obtained compounds V may then be reacted with a monosubstituted piperazine, to produce the desired type I compounds. Such alkylation reactions are carried out by means of conventional methods, which are well known to those experienced in the art. In general, in reactions of this type, alkylation is carried out in an aprotic solvent (for example, acetonitrile, dimethylformamide, toluene, dioxane or tetrahydrofuran), or a protic solvent (for example, ethanol, *n*-butanol), or else in the absence of a solvent, and in the presence of at least one base (for example, triethylamine, diisopropylethylamine, pyridine, 4-dimethylaminopyridine or potassium carbonate), at a temperature within the interval room temperature - 180°C.

The type IV and type V intermediate compounds discussed above may also be prepared by means of the alkylation of an initial $ArCH_2Ar'$ compound, in the form of its metallic carbanion (which is initially obtained through the use, for example, of a base such as butyl lithium or another suitable lithium complex, or else from another suitable base which is derived from an alkaline metal), with a compound of either formula X-Z-CH_2-OPrG or X-Z-CH_2-X, where X and Z have the meanings assigned above, and the term PrG represents a protecting group (for example, o-tetrahydropyranyl), which is removed after the alkylation process has been completed. In cases where the same alkylation technique is employed for an ArCHAr' compound with the successive type VI compound, where X represents a leaving group, of the type described above:



(VI)

it becomes possible to carry out the direct preparation of the type I product compound.

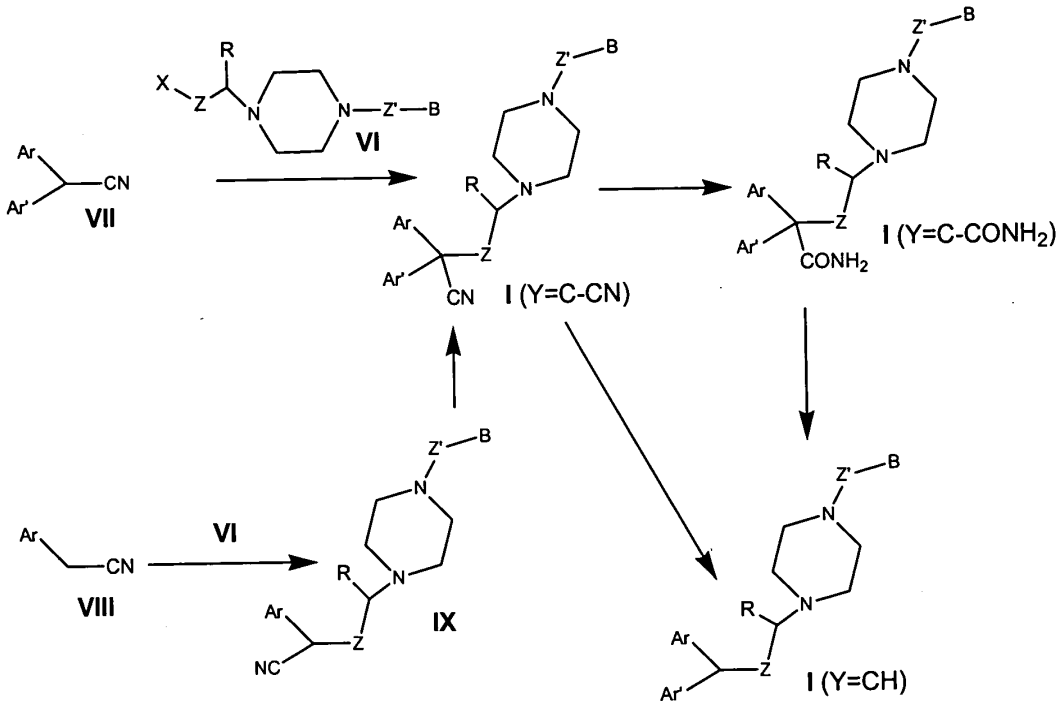
Type VI compounds may be prepared easily from initial type VI compounds which possess a COOAk group in the place of the terminal group -CH_2-X. In such cases, conventional reduction procedures (for example, reduction with lithium aluminum hydride

or other suitable metallic hydride complexes) may be employed to generate the corresponding type VI compounds with X = OH, which may then, in turn, be converted (once again, through the use of conventional procedures) into type VI compounds with X= a leaving group. The initial esters may be prepared either through the use of the well-known Michael reaction, or, alternatively, by means of nucleophilic substitution reactions which involve a monosubstituted piperazine and a suitable 2,3-unsaturated ester or a 2-alloester.

An alternative procedure for use in the production of type VI compounds involves the alkylation of suitable monosubstituted piperazine derivatives with a compound of either formula X-CH(R)-Z-OPrG or formula X-Z-CH_2-X, where X and Z have the meanings assigned above, and the term PrG represents a protecting group (for example, O-tetrahydropyranyl), which is removed after the alkylation process has been completed.

Compounds of type I (the subject of the present invention) where Y represents a C-CN, C-$CONH_2$ or CH group, and in which the terms Ar, Ar', R, B, Z and Z' are defined as above, may generally be prepared via synthetic Scheme 2:

Scheme 2



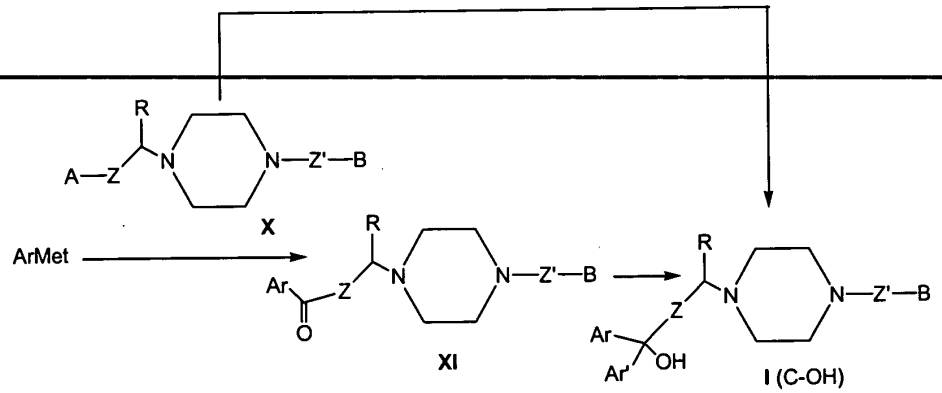
The type VII intermediate compounds, which are, in general, either commercially available or readily obtainable through the use of conventional synthesis procedures, can be converted to type I product compounds, with Y = C-CN, by means of the alkylation of the corresponding carbanion with a suitable type VI piperazine derivative (*Il Farmaco,* **1995**, 50, 505). Such alkylation procedures are carried out through the use of a base (for example, butyl lithium, sodium amide, sodium hydride, lithium diisopropylamide, lithium bis(trimethylsilyl)amide, or another appropriate substance of this type, which should be well known to those of skill in the art), in a suitable aprotic solvent (such as, for instance, toluene, tetrahydrofuran, dimethoxyethane, dioxane, diglyme, etc.), at a temperature within the interval -20°C to reflux temperature. The obtained type I compounds, where Y = C-CN, may be easily transformed, through the use of conventional procedures (partial acid hydrolysis, carried out with an aqueous acid such as 70% sulfuric acid or a suitable Lewis acid, within the temperature interval room temperature - 80°C; March, *Advanced Organic Chemistry,* Wiley Intersecience Ed., **1992**, 887) into type I product compounds wherein Y = C-$CONH_2$. An alternative procedure, for use when the obtained type I compounds are of the type in

whichY = CH, comprises acid hydrolysis carried out under more severe conditions (for example, 70% sulfuric acid under reflux conditions).

A final variant of this procedure involves the use of the same C-alkylation procedure which was described above, carried out with suitable type VI piperazine derivatives and type VIII $ArCH_2CN$ compounds (which are normally either commercially available or readily obtainable through the use of conventional synthesis procedures), yielding type IX intermediate compounds, which, in turn, can be subjected to arylation with Ar'-LG compounds (where LG represents a leaving group, which may comprise a chlorine, bromine or fluorine atom) this normally involves a phase-transfer reaction carried out in the presence of a base (for example, 50% sodium hydroxide; *Tetrahedron Letter,* **1969**, 673) and of a suitable catalyst (for example, triethylbenzylammonium chloride) in an appropriate solvent (such as toluene), within the temperature interval room temperature - reflux temperature. In such reactions, the aryl group employed must be sufficiently active in aromatic nucleophilic substitution, carried out in the presence of electron-attractor groups in the proper position and/or electron-poor heterocycles (*Chem. Rev.,* **1951**, 49, 273)

The type I compounds of the present invention where Y represents a C-OH group, and Ar, Ar', R, B, Z and Z' are defined as above, are typically prepared as indicated below, in synthesic Scheme 3:

Scheme 3

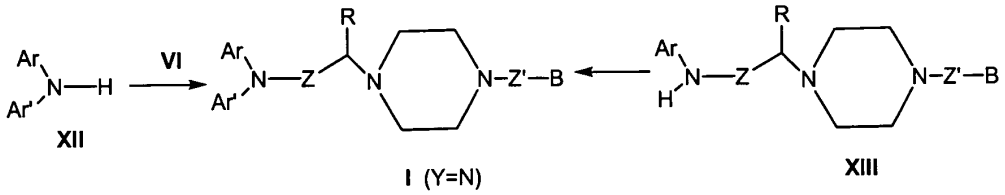


The metallic aryl derivatives of the type ArMet, where Met stands for a metal (for example, lithium or halogenated magnesium) which are prepared by means of the reacting of butyl lithium or magnesium with an aryl-Br or aryl-I compound, in either tetrahydrofuran or

diethyl ether, within the temperature interval -70°C - reflux temperature, are subsequently reacted, in the same solvents, within the temperature interval -20°C - reflux temperature, with type X derivatives (which can be readily synthesized via conventional procedures), where A can represent a carboxyl, cyano or $CONH_2$ group, to obtain type I product where Ar = Ar'. In such compounds, X is preferably, either an alkyl piperazine propionate or an alkyl piperazine acetate. In certain cases, it is possible to carry out the direct lithiumizing of the aryl species - for example, in cases in which the Ar contains an *ortho*-dimethylaminocarbonyl or methoxy substituent. Compuonds X in which A represents a group $(CH_3O)(CH_3)NC(O)$ (A', Weinreb amide) it is possible to carry out a two-stage reaction, with the isolation of the type XI ketone intermediate. This type XI intermediate compound is then reacted with Ar'Met an alcoholic type I compound, which also contains different aryl groups.

The type I product compounds of the present invention in which Y is a nitrogen atom are typically prepared as indicated below in synthetic Scheme 4:

Scheme 4



The type XII intermediate compounds may be transformed, through *N*-alkylation, into the corresponding aza-anions, when reacted with the appropriate type VI compounds (see description above). This alkylation reaction is carried out in the presence of a base (for example, butyl lithium, sodium amide, sodium hydride, lithium diisopropylamide, lithium bis(trimethylsilyl)amide, or another suitable base, well known to those of skill in the art), in a suitable aprotic solvent such as toluene, tetrahydrofuran, dimethoxyethane, dioxane or diglyme, at a temperature within the interval -20°C - reflux temperature.

The type XII intermediate compounds are either commercially available or readily synthesized through the use of procedures described in the literature, such as, for example, a nucelophilic substitution reaction which involves an Ar-NH_2 compound and an Ar'-LG compound (where LG is a leaving group such as bromine, chlorine, fluorine, iodine or

trifluoromethanesulfonyloxy). This nucleophilic reaction is conducted either with or without a catalyst, and is generally carried out in the presence of a suitable base, such as sodium carbonate, lithium diisopropylamide, sodium tert-butoxide, etc.

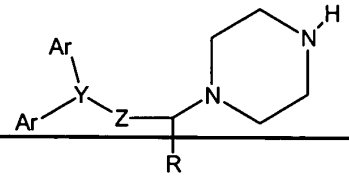
The metallic catalyst employed in such reactions may be selected from a wide range of possible substances, including, for example, copper, or copper (I) iodide, bromide or oxide (*Tetrahedron*, **1984**, 40, 433), nickel-base catalysts (cf. *J. Org. Chem.*, **1975**, 40, 2267), or palladium dichloride, palladium diacetate, palladium *tetrakis*-(triphenylphosphine), bis(diphenylphosphine)palladium dichloride, palladium dibenzylidene acetone, bis(diphenylphosphinoferrocene)palladium dichloride (*Synlett.*, **1996**, 329; *J. Org. Chem.*, **1997**, 62, 1568; **1997**, 62, 1268; **1997**, 62, 1264). Reactions of this type are carried out at fusion temperature, either without a solvent or with an appropriate adapted solvent (for example, dimethylacetamide, dimethylformamide, dioxane, toluene or tetrahydrofuran), at a temperature within the interval room temperature - reflux temperature, in the presence of at least one ligand (for example, triphenylphosphine, tri-o-tolyl-phosphine, bis(diphenylphosphino)ferrocene, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, or another suitable, commercially available phosphinic ligand.

An alternative procedure for use in the obtaining of type I product compounds, which is particularly effective in cases in which one or both of the aryl groups involved contains a nitro group, consists in the arylation of the type XIII intermediate amine compound, through the use of the same procedures as those described above, in the discussion of the preparation of the type XII intermediates.

The type XIII intermediate compounds can be produced through the use of conventional procedures which are well-known to experienced practitioners in this field, and which generally comprise the alkylation of an Ar-NH_2 aniline derivative with a suitable type VI compound, in the presence of a suitable solvent (for example, n-butanol), either at the highest boiling temperature or at the fusion point. Alternatively, if the arylic function has been adapted successfully to the desired end (for example, in cases in which, as described above, there is a sufficient level of activity in regard to aromatic nucleophilic substitution), it is possible to carry out the procedure in question by means of the reacting of an Ar-LG (in which the term LG is defined as above) with a suitable omega-aminoalkylpiperazine

derivative. Such reactions may be carried out at fusion temperature, without a catalyst, either in a suitable solvent (such as, for instance, n-butanol, dimethylformamide or dimethylacetamide), within the temperature interval room temperature - reflux temperature. Such reactions may also be catalyzed, as described above, in the case of the preparation of the type XII intermediate compounds.

In cases in which the term B represents a lower aryl or heteroaryl, the preparation of the type I product compounds may be carried out as described above or, alternatively, the type I compounds may be produced through the use of piperazine derivatives, in which the term Z'-B represents a protective group (such as, for example, *tert*-butoxycarbonyl, benzyloxycarbonyl, benzyl, or another suitable substance selected from among the various amine protectors described in: Green, *"Protective Groups in Organic Synthesis"*, Wiley Interscience, New York (1991). Through the use of synthesis procedures of the same general type as those described above, a type I product compound is obtained, in which the term Z'-B represents a protective group of the type noted above. Simple, conventional deprotection procedures are employed in the preparation of type XIV compounds, which may then be subjected to alkylation with a suitable halogenized arylalkyl or heteroalkyl, to yield type I compounds of the type specified in the present invention.



XIV

EXAMPLE 1

1-(3,3-diphenylpropyl)-4-(2-methoxyphenyl)piperazine hydrochloride.

a) <u>1-(3,3-diphenylpropionyl)-4-(2-methoxyphenyl)piperazine (lA)</u>

To an inital solution comprising 1.13 g of 3,3-diphenylpropionic acid and 1.06 g of 1-(2-methoxyphenyl) piperzine in 25 mL of *N,N*-dimethyl formamide, maintained, under constant agitation, at a temperature within the range of 0-5°C, there was added, in succesion,

0.9 mL of 93%,diethyl cyanophosphonate and 0.77 mL triethylamine. The resultant solution was then subjected to agitiation at room temperature, for 5 hr, followed by its decanting into 250 mL of water, with subsequent extraction using ethyl acetate. The obtained organic phase is then rinsed with water, dried with anhydrous sodium sulfate, and then subjected to evaporation under a desiccating vacuum. The obtained oily residue is then subjected to flash-chromatography purification (chloroform - ethyl acetate 9:1). This procedure generates the organic substance represented above, in the title of this Example, at the theoretical yield rate.

^{1}H-NMR ($CDCl_3$, δ): 7.15 - 7.35 (m, 10H, phenyl protons); 6.75 - 7.05 (m, 4H, methoxyphenyl protons); 4.69 (t, 1H, CH); 3.85 (s, 3H, OCH_3); 3.67 - 3.77 (m, 2H, piperazine protons); 3.47-3.67 (m, 2H, piperazine protons); 3.10 (d, 2H, $CH_2C(O)$), 2.83 - 2.93 (m, 2H, piperazine protons; 2.67 - 2.77 (m, 2H, piperazine protons).

b) 1-(3,3-diphenylpropyl)-4-(2-methoxyphenyl)piperazine hydrochloride

To an initial solution comprising 2.0 g of the compound described above, in Example 1A, in 45 mL of anhydrous tetrahydrofuran, maintained under constant agitation at room temperature, there was added 0.44 g of lithium aluminum hydride. The obtained reaction mixture was then subjected to agitation at room temperature, for a period of 24 hr, and then for 2.5 hr under reflux conditions. The mixture was then cooled, and 5 mL ethyl acetate was added cautiously, followed by 5 mL ethanol. Next, the mixture was decanted into 225 mL of water, followed by extraction with ethyl acetate. The obtained organic phase was rinsed with water, desiccated over anhydrous sodium sulfate, and then subjected to vacuum evaporation. The crude product obtained by this means was then subjected to purification, via flash chromatography (petroleum ether - ethyl acetate 7:3). The residual matter obtained by means of the evaporation of the recovered fractions was then dissolved in ethyl acetate, and to the resultant solution there was added 1 molar equivalent HCl (2 N solution, in ethanol). The product material described above, in the title, was then crystallized and isolated, via filtration processing, at a yield rate of 39% (0.83 g of product obtained). M.p. 143-149°C.

^{1}NMR ($CDCl_3$, δ): 12.75 - 13.10 (sa, 1H, NH+); 7.15 - 7.35 (m, 10H, phenyl protons); 6.80 - 7.12 (m, 4H, methoxyphenyl protons), 3.99 (t, 1H, CH), 3.85 (s, 3H, OCH_3);

3.38 - 3.70 (m, 6H, piperazine protons, $C\underline{H_2}NH+$), 2.85 - 3.15 (m, 4H, piperazine protons), 2.65 - 2.82 (m, 2H, $C\underline{H_2}CH$).

EXAMPLE 2

1-(3,3-diphenylpropyl)-4-[5-(2,3-dihydro-l,4-benzodioxinyl)]piperazine methane sulphonate

a) 1-(3,3-diphenylpropionyl)-4-[5-(2,3-dihydro-l,4-benzodioxinyl)]-piperazine (2A)

This product material was obtained in accordance with the method provided above, in Example lA), with the sole difference that the 1-(2-methoxyphenyl)piperazine raw material was replaced here by 1-[5-(2,3-dihydro-l,4-benzodioxinyl)piperazine. The obtained crude product material was subjected here to purification with the aid of flash chromatography (chloroform - ethyl acetate 8:2); the product yield rate here was 85%.

^{1}H-NMR ($CDCl_3$, δ): 7.15 -7.35 (m, 10H, phenyl protons), 6.74 (dd, 1H, benzodioxane H7), 6.60 (dd, 1H, benzodioxane H6), 6.40 (dd, 1H, benzodioxane H8), 4.68 (t, 1H, CH), 4.15 - 4.35 (m, 4H, OCH_2CH_2O); 3.65 - 3.75 (m, 2H, piperazine protons); 3.40 - 3.50 (m, 2H, piperazine protons), 3.10 (d, 2H, $CH_2C(O)$), 2.85 - 2.95 (m, 2H, piperazine protons); 2.65 - 2.75 (m, 2H, piperazine protons).

b) 1-(3,3-diphenylpropyl)-4-[5-(2,3-dihydro-l,4-benzodioxinyl)]piperazine methane sulphonate

This product material was obtained in accordance with the method provided above, in Example l), with the sole exception that compound 2A was employed in place of compound lA. The residual material from the chromatography column was dissolved in ethyl acetate and, followed by the addition of one molar equivalent of methane sulfonic acid (0.5 M solution, in ethyl acetate). After holding the resultant solution overnight at 3°C, the crystallized product material was recovered, at a yield rate of 21 %,via filtration procedures. M.p. 194-195°C.

^{1}H-NMR (DMSO-d_6, δ): 9.35 - 9.55 (sa, 1H, NH+), 7.12 - 7.40 (m, 10H, phenyl protons);, 6.75 (dd, 1H, benzodioxane H7), 6.50 - 6.58 (2dd, 2H, benzodioxane H6, H8),

4.18 - 4.28 (m, 4H, OCH_2CH_2O), 4.05 (t, 1H, CH); 3.45 - 3.68 (m, 4H, piperazine protons), 2.80 - 3.30 (m, 6H, piperazine protons, $CHCH_2C\underline{H}_2$); 2.45 - 2.55 (m, 2H, $CHC\underline{H}_2CH_2$); 2.30 (s, 3H, CH_3S).

EXAMPLE 3

1-[3,3-*bis*-(4-nitrophenyl)propyl]-4-(2-methoxyphenyl)piperazine dihydrochloride·0.8 H_2O

a) 1-[3,3-*bis*-(4-nitrophenyl)propionyl]-4-(2-methoxyphenyl)-piperazine (3A)

This product material was obtained in accordance with the method provided above, in Example 1A, with the sole exception that the initial 3,3-diphenylpropionic acid was replaced here by 3,3-*bis*-(4-nitrophenyl)propionic acid (prepared in accordance with the method described by Pfeiffer et al, *Annalen*, **1953**, *581*, 149). In addition, extraction was carried out here with chloroform rather than ethyl acetate. The obtained crude product material was purified by means of crystallization from 80% ethanol. The obtained solid (yield rate: 48%) exhibited a melting temperature range of 159-163°C.

^{1}H-NMR ($CDCl_3$, δ): 8.18 (dd, 4H, nitrophenyl H3, 5), 7.42 (dd, 4H, nitrophenyl H2, 6), 6.80 - 7.14 (m, 4H, methoxyphenyl aromatics), 4.97 (t, 1H, CH), 3.86 (s, 3H, OCH_3), 3.67 - 3.78 (m, 2H, CH_2), 3.58 - 3.67 (m, 2H, $CON(CH\underline{H})_2$ equatorials), 3.16 (d, 2H, $CON(C\underline{H}H)_2$ axials), 2.90 - 3.07 (m, 4H, remaining piperazine protons).

b) 1-[3,3-*bis*- 4-nitrophenyl)propyl]-4-(2-methoxyphenyl)piperazine dihydrochloride 0.8 H_2O

To a solution of 0.49 g of Compound 3A, in 6 mL anhydrous tetrahydrofuran, subjected to constant agitation under a nitrogen atmosphere, there was added, at a temperature of 0-5°C, 1.25 mL of borane-dimethyl sulfide (2 M solution, in tetrahydrofurane). The obtained mixture was then subjected to reflux agitation for 4 hr, followed by cooling to 0°C, followed by the addition of 1 mL methanol, and, subsequently, 0.5 hr of agitation within the temperature interval 20-25°C. Next, 0.5 mL hydrochloric acid (4 N solution, in isopropanol) was added. The resultant mixture was then subjected to 1 hr of reflux agitation, diluted with 20 mL methanol, and evaporated until completely dehydrated,

under a vacuum. The obtained residual material was then renewed with 10 mL of water, and the resultant mixture was rendered basic by the addition of 1 N sodium hydroxide. This was followed by extraction with 3 x 5 mL chloroform. The reunited organic phases were then rinsed with water, dried with anhydrous sodium sulfate, and evaporated until dry under a vacuum. The residue was then dissolved in 18 mL methanol, followed by acidification of the obtained solution with 4 N hydrochloric acid in excess isopropanol. After 3 hr holding at a temperature of 0°C, the crystallized product material was recovered via filtration. The yield rate in this case was 55.7% (0.31 g product). The obtained product material exhibited a melting point range of 191-194°C, and contained 0.8 moles of water.

^{1}H-NMR ($CDCl_3$, δ): 11.25 - 11.45 (sa, 1H, NH+), 8.20 (dd, 4H, nitrophenyl H3, 5), 7.70 (dd, 4H, nitrophenyl H2, 6), 6.85 - 7.07 (m, 4H, methoxyphenyl aromatics), 5.85 - 6.18 (sa, 2.6H, H_2O and NH+), 4.54 (t, 1H, CH), 3.77 (s, 3H, OCH_3), 3.55 - 3.65 (m, 4H, piperazine protons), 3.07 - 3.25 (m, 4H, piperazine protons), 2.90 - 3.07 (m, 2H, $CHCH_2C\underline{H_2}N$), 2.63 - 2.80 (m, 2H, $CHC\underline{H_2}CH_2N$).

EXAMPLE 4

1-[3,3-bis-(4-methoxyphenyl)propyl]-4-(2-methoxyphenyl)piperazine dihydrochloride

a) 1-[3,3-*bis*- 4-methoxyphenyl)propionyl]-4-(2-methoxyphenyl)piperazine hydrochloride (4A)

This product material was obtained in accordance with the method provided above, in Example lA, with the sole exception that, in place of the 3,3-diphenylpropionic acid, 0.57 g of 3,3-*bis*-(4-methoxyphenyl)propionic acid (prepared in accordance with the method described in Klemm, L. H., *J. Org. Chem.*, **1958**, *23*, 344) was employed here. In addition, extraction was conducted here through the use of diethyl ether, instead of ethyl acetate, and the obtained extract, following its drying over anhydrous sodium sulfate, was subjected to acidification with hydrochloric acid (3 N solution, in diethyl ether). The precipitate was then recovered through filtration, and was recrystallized with acetone. product material yield rate here was 65.5% (0.65 g); the obtained product possessed a melting point range of 175-179°C.

^{1}H-NMR (DMSO-d_6, d): 9.50 (sa, 1H, NH+), 7.15 - 7.25 (m, 4H, methoxyphenyl aromatics of the AA'BB' system), 6.88 - 7.25 (m, 4H, methoxyphenyl protons), 6.76 - 6.85 (m, 4H, methoxyphenyl aromatics of the AA'BB' system), 4.38 (t, 1H, CH), 3.82 (s, 3H, OCH_3), 3.55-3.80 (m, 4H, piperazine protons), 3.67 (s, 6H, 2 OCH_3), 2.88 - 3.15 (m, 6H, piperazine protons, $C(O)CH_2$).

b) 1-[3,3-*bis*-(4-methoxyphenyl)propyl]-4-(2-methoxyphenyl)piperazine dihydrochloride

This product material was obtained in accordance with the procedure described above, in Example 3, with the sole exception that Compound 4A was employed here in place of Compound 3A. In addition, extraction was carried out here with ethyl acetate rather than chloroform. The obtained residue was dissolved in diethyl ether; then, after treatment with carbon, the resultant solution was subjected to acidification with excess hydrochloric acid (3 N solution, in diethyl ether). After 3 hr, the precipitate was recovered by means of filtration; this product material exhibited a melting point range of 163-171°C.

^{1}H-NMR (DMSO-d_6, δ): 8.80 - 9.60 (sa, 2H, NH+), 7.18 - 7.30 (m, 4H, methoxyphenyl aromatics of the AA'BB' system), 6.80 - 7.05 (m, 8H, methoxyphenyl protons and other methoxyphenyl aromatics of the AA'BB' system), 3.92 (t, 1H, CH); 3.78 (s, 3H, OCH_3), 3.71 (s, 6H, 2 OCH_3), 3.35 - 3.62 (m, 4H, piperazine protons), 3.03 - 3.25 (m. 4H, piperazine protons), 2.85 - 3.03 (m, 2H, CH_2CH_2CH), 2.42 - 2.52 (m, 2H, CH_2CH_2CH).

EXAMPLE 5

1-[N-N-*bis*-(2-pyridyl)-2-aminoethyl]-4-(2-methoxyphenyl)piperazine hydrochloride

To a solution of 1.71 g *bis*-(2-pyridyl)amine in 50 mL toluene, under constant agitation, there was added, at room temperature, 0.55 g of 95% sodium amide, followed by 2.54 g of 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine. The obtained reaction mixture was then subjected to reflux agitation for 24 hr, followed by cooling to room temperature and subsequent careful dilution with 10 mL methanol. Then, after 15 min of agitation, 20 mL water and 20 mL ethyl acetate were added. Then, after 10 min of further agitation, phase

separation was carried out, and the obtained aqueous phase was subsequently re-extracted with ethyl acetate. The reunited organic phases were then rinsed with water, subjected to anhydriding over sodium sulfate, and then evaporated to complete dryness under a vacuum. The obtained crude product material was then purified through the use of flash chromatography (gradient of petroleum ether-ethyl acetate-2.2 N solution of ammonia in methanol from 6:4:0.2 to 4:6:0.2). The recovered fraction was then evaporated to complete dryness, yielding 2.51 g (64.5% yield rate) of basic product material. This material was subsequently dissolved in 45 mL ethyl acetate, to which was added 1 molar equivalent hydrochloric acid (1 M solution in ethanol). Overnight holding at a temperature of 0°C yielded the end-product material, in crystalline form; this substance was found to possess a melting point range of 218-220°C.

^{1}H-NMR (DMSO-d_6, δ): 8.40 (dd, 2H, pyridine H6), 7.74 (ddd, 2H, pyridine H4), 7.28 (dd, 2H, pyridine H3), 6.90 - 7.15 (m, 6H, pyridine H5, phenyl protons), 4.58 (t, 2H, $PyNCH_2$), 4.35 - 5.15 (sa, 1H, NH^+), 3.80 (s, 3H, OCH_3); 2.95 - 3.35 (m, 10H, piperazine protons and $PyNCH_2C\underline{H_2}$).

EXAMPLE 6

1-[3-cyano-3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine

To a suspension of 0.21 g of 95% sodium amide in 2 mL 1,2-dimethoxyethane was added, dropwise, a solution of 0.78 g of 2,2-*bis*-(2-pyridyl)acetonitrile (prepared as described in *Heterocycles*, **1995**, *40*, 757) in 8 mL 1,2-dimethoxyethane, with constant mixture agitation, and under a nitrogen atmosphere, at room temperature. After 1 hr, there was added, also in dropwise fashion, 1.02 g of 1-(2-chloroethyl)-4-(2-methoxy-phenyl)piperazine dissolved in 4 mL of 1,2-dimethoxyethane. The resultant reaction mixture was then subjected to 20 hr reflux agitation, followed by cooling to room temperature, and was then decanted cautiously into 40 g of ice, diluted with water, and extracted with ethyl acetate. The reunited organic phases were then rinsed with water, subjected to drying over sodium sulfate, and then evaporated thoroughly under a vacuum. The obtained crude product material was then purified through the use of flash chromatography (ethyl acetate-methanol

gradient from 10:0 to 9:1). The recovered fraction was then evaporated to complete dryness, yielding 1.13 g product material (yield rate: 68.4%).

^{1}H-NMR ($CDCl_3$, d): 8.60 (dd, 2H, pyridine H6), 7.58 - 7.73 (m, 4H, pyridine H3, 4), 7.22 (ddd, 2H, pyridine H5), 6.83 - 7.03 (m, 4H, methoxyphenyl aromatics), 3.84 (s, 3H, OCH_3), 2.85 - 3.08 (m, 6H, piperazine protons, $CC\underline{H_2}CH_2N$), 2.55 - 2.70 (m, 6H, piperazine protons, $CCH_2C\underline{H_2}N$).

EXAMPLE 7

1-[3-cyano-3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine dihydrochloride

This product material was prepared as described above, in Example 6, with the sole exception that the 2,2-*bis*-(2-pyridyl)acetonitrile was replaced here with 1.86 g of 2-phenyl-2-(2-pyridyl)acetonitrile (prepared as described in *Helv. Chim. Acta*, **1944**, *27*, 1748). The obtained crude product material was purified by means of flash chromatography (ethyl acetate- petroleum ether 6:4). After subsequent thorough evaporation of the recovered fraction, this procedure yielded 3.39 g (yield rate: 86%) of basic product material. This initial product material was subsequently dissolved in 20 mL ethanol, to which was then added 6 mL of hydrochloric acid (5 M solution, in isopropanol). Finally, after overnight holding at room temperature, 3.45 g of crystalline end-product material was recovered via filtration; this material exhibited a melting-point range of 228-230°C.

^{1}H-NMR (DMSO-d_6, δ): 11.50 - 11.75 (sa, 1H, NH+); 8.65 (dd, 2H, pyridine H6), 8.25 - 8.60 (sa, 1H, NH+), 8.40 (ddd, 2H, pyridine H4), 7.45 - 7.60 (m, 7H, pyridine H3, 5, phenyl protons), 6.85 - 7.10 (m, 4H, methoxyphenyl aromatics), 3.77 (s, 3H, OCH_3), 3.00 - 3.75 (m, 12H, piperazine protons and CH_2CH_2).

EXAMPLE 8

1-[3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine

An initial mixture comprising 2.44 g of the compound from Example 6 and 12 mL of 70% sulfuric acid was subjected to agitation for 1.5 hr, at a temperature of 125°C. The obtained reaction mixture was then cooled to room temperature, followed by careful

decanting into 100 g of ice, dilution with water, alkalization with 35% sodium hydroxide, and extraction with ethyl acetate (3 x 40 mL). The reunited organic phases were then rinsed with water, subjected to drying over anhydrous sodium sulfate, and then evaporated to complete dryness under a vacuum. The obtained crude product material was then purified by means of flash chromatography (ethyl acetate-2.2 N solution of ammonia in methanol, 9.6:0.4). The recovered fraction was then evaporated to complete dryness, yielding 1.87 g of end product material (yield rate: 82%).

^{1}H-NMR ($CDCl_3$, δ), 8.55 (dd, 2H, pyridine H6), 7.58 (ddd, 2H, pyridine H4), 7.36 (dd, 2H, pyridine H3), 7.10 (ddd, 2H, pyridine H5), 6.79 - 7.03 (m, 4H, methoxyphenyl aromatics), 4.37 (t, 1H, CH), 3.84 (s, 3H, OCH_3), 2.95 - 3.12 (m, 4H, piperazine protons), 2.55 - 2.73 (m, 4H, piperazine protons), 2.30 - 2.55 (m, 4H, CCH_2CH_2N).

EXAMPLE 9

1-[3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine and

EXAMPLE 10

1-[3-carbamoyl-3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)-piperazine

An initial mixture comprising 1.26 g of the compound from Example 7 and 6.2 mL of 70% sulfuric acid was agitated for 40 min at 125°C. The obtained reaction mixture was then cooled to room temperature, decanted cautiously into 60 g of ice, diluted with water, alkalinized with 35% sodium hydroxide, and extracted with ethyl acetate (2 x 60 mL). The reunited organic phases were then rinsed with water and anhydrified over anhydrous sodium sulfate, followed by evaporation to dryness under a vacuum. The obtained crude product material was then purified, with the aid of flash chromatography (ethyl acetate-petroleum ether- 2.7 *N* solution of ammonia in methanol gradient, from 5:5:0.5 to 8:2:0.5). Subsequent vacuum evaporation of the less polar fractions resulted in the production of 0.25 g of the compound from Example 9.

^{1}H-NMR ($CDCl_3$, δ): 8.59 (dd, 1H, pyridine H6), 7.54 (ddd, 1H, pyridine H4), 7.08 - 7.41 (m, 7H, pyridine H3, 5, phenyl aromatics), 6.82 - 7.07 (m, 4H, methoxypheyl

aromatics), 4:18 (t, 1 H, C<u>H</u>CH$_2$); 3.85 (s, 3H, OCH$_3$), 3.00 - 3.15 (m, 4H, piperazine protons), 2.25 - 2.73 (m, 8H, piperazine protons and CH$_2$CH$_2$).

From the evaporation of the more polar fractions, 0.78g of product material was obtained here; this material comprised the compound from Example 10 in oily form. This material, after crystallization with acetonitrile and filtration recovery, comprised 0.35 g of a solid with a melting point range of 156-164°C.

^{1}H-NMR (CDCl$_3$, δ): 9.20 - 9.40 (sa, 1H, CONH$_2$), 8.55 (dd, 1H, pyridine H6), 7.60 (ddd, 1H, pyridine H4), 7.10 - 7.35 (m, 7H, pyridine H3, 5, phenyl aromatics), 6.80 - 7.05 (m, 4H, methoxyphenyl aromatics), 5.60 - 5.75 (sa, 1H, CONH$_2$), 3.83 (s, 3H, OCH$_3$), 2.15 - 3.15 (m, 12H, piperazine protons and CH$_2$CH$_2$).

EXAMPLE 11

1-[*N*-(2-nitrophenyl)-N-(2-pyridyl)-2-aminoethyl]-4-(2-methoxyphenyl)-piperazine

An initial mixture comprising 0.43 g of 1-[*N*-(2-nitrophenyl)-2-aminoethyl]-4-(2-methoxyphenyl)piperazine (prepared as described in U.S. 3, 472, 854), 0.19 g of 2-bromopyridine, 0.17 g of anhydrous potassium carbonate and 0.01 g of powdered copper was heated to 100°C, and held at that temperature for 3 hr, followed by the addition of another 0.138 g of 2-bromopyridine, and the heating of the mixture to 160°C, at which temperature it was held for 24 hr. This was followed by the cooling of the mixture to room temperature and extraction with ethyl acetate (2 x 20 mL). The reunited organic phases were then rinsed with water, dried over sodium sulfate, and subjected to vacuum evaporation until completely dry. The obtained crude product material was next purified through the use of flash chromatography (ethyl acetate-petroleum ether 7:3). The recovered fraction, after evaporation to dryness, yielded 0.25 g of Compound 11 (yield rate: 52%).

^{1}H-NMR (CDCl$_3$, δ): 8.12 (dd, 1H, pyridine H6); 7.98 (dd, 1H, nitrophenyl H3); 7.52 - 7.70 (m, 2H, aromatics), 7.30 - 7.50 (m, 2H, aromatics), 6.79 - 7.03 (m, 4H, methoxyphenyl aromatics), 6.65 (dd, 1H, pyridine H5), 6.33 (dd, 1H, pyridine H3), 4.08 (t, 2H, CH$_2$NPy), 3.84 (s, 3H, OCH$_3$), 2.90 - 3.05 (m, 4H, piperazine protons), 2.80 (t, 2H, C<u>H$_2$</u>CH$_2$NPy), 2.60 - 2.75 (m, 4H, piperazine protons).

EXAMPLE 12

1-[3-cyano-3-(2-nitrophenyl)-3-phenylpropyl]-4-(2-methoxyphenyl) piperazine.

a) 1-(3-cyano-3-phenylpropyl)-4-(2-methoxyphenyl)piperazine (12A)

This product material was synthesized here through the use of the method described above, in Example 6, with the replacement of 2,2-*bis*-(2-pyridyl)acetonitrile with 0.59 g phenylacetonitrile, and substituting toluene for 1,2-dimethoxyethane. The obtained reaction mixture was subjected to agitation for 3.5 hr, at a temperature of 80°C. The obtained crude product material was then purified via flash chromatography (ethyl acetate-petroleum ether 6:4). The recovered fraction was then evaporated to dryness, yielding 0.96 g of Compound 12 (yield rate: 57.3%).

^{1}H-NMR ($CDCl_3$, δ): 7.35 - 7.45 (m, 5H, phenyl aromatics), 6.79 - 7.03 (m, 4H, methoxyphenyl aromatics), 4.08 (t, 1H, CH), 3.86 (s, 3H, OCH_3), 3.05 m- 3.20 (m, 4H, piperazine protons), 2.38 - 2.70 (m, 6H, piperazine protons, 2H of CH_2CH_2), 1.95 - 2.35 (m, 2H, 2H of CH_2CH_2).

b) 1-[3-cyano-3-(2-nitrophenyl)-3-phenylpropyl]-4-(2-methoxyphenyl)-piperazine

An initial mixture comprising 0.24 g of the compound from Example12A, 0.11 g 2-chloro-nitrobenzene, 0.5 mL 50% sodium hydroxide 0.02 g triethyl benzyl ammonium chloride, and 0.5 mL toluene was agitated for 6 hr at 60°C, followed by cooling of the mixture to room temperature, dilution with 20 mL of water, and extraction with ethyl acetate (2 x 20 mL). The reunited organic phases were then rinsed with water, subjected to dried over sodium sulfate, and evaporated to dryness under a vacuum. The obtained crude product material was purified by means of flash chromatography (ethyl acetate-petroleum ether 5:5). The recovered fraction was then evaporated to complete dryness, yielding 0.12 g of the title compound (yield rate: 36%). This initial product material was then dissolved in methylene chloride, evaporated under a vacuum, and subjected to vacuum (1 mmHg) drying. The obtained end-product material possessed a melting point range of 61-64°C.

^{1}H-NMR ($CDCl_3$, δ): 8.05 (dd, 1H, nitrophenyl H3), 7.50 - 7.73 (m, 3H, nitrophenyl H 4, 5, 6), 7.20 - 7.35 (m, 5H, phenyl aromatics), 6.79 - 7.03 (m, 4H, methoxyphenyl aromatics), 3.84 (s, 3H, OCH_3); 2.95 - 3.15 (m, 5H, piperazine protons, C<u>H</u>HCH_2N), 2.35 - 2.75 (m, 7H, piperazine protons, CH<u>H</u>C<u>H</u>$_2$N).

EXAMPLE 13

1-[3-carbamoyl-3-(2-nitrophenyl)-3-phenylpropyl]-4-(2-methoxyphenyl)-piperazine

This product was obtained through the use of the method described above, in Example 8, with the exception that 0.21 g of Compound 12, rather than Compound 6, was initially held at 125°C for 105 min. After the usual processing stages, the obtained crude product material was purified through the use of flash chromatography (ethyl acetate-methanol 95:5). The recovered fraction was then evaporated, yielding 0.1 g of Compound 13, in oily form (yield rate: 46%).

^{1}H-NMR ($CDC1_3$, δ): 7.75 - 7.82 (m, 1H, nitrophenyl H3), 7.55 - 7.80 (m, 1H, $CONH_2$), 7.25 - 7.50 (m, 7H, phenyl aromatics, nitrophenyl H 4, 5), 7.05 - 7.15 (m, 1H, nitrophenyl H6), 6.79 - 7.03 (m, 4H, methoxyphenyl aromatics), 5.30 - 5.55 (m, 1H, $CONH_2$), 3.84 (s, 3H, OCH_3), 3.00 - 3.15 (m, 4H, piperazine protons), 2.25 - 2.95 (m, 8H, piperazine protons, CH_2CH_2).

EXAMPLE 14

1-[3-hydroxy-3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine

To an initial solution comprising 0.17 mL of 2-bromopyridine in 6 mL tetrahydrofurnace, agitated under a nitrogen atmosphere, at a temperature of -50°C, there was added, in a dropwise manner, over a period of 5 min, 0.72 mL butyl lithium (2.5 M solution, in hexane). After 6 min holding at -55°C, there was added, in dropwise fashion, over a period of 10 min, a solution comprising 0.5 g of 3-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl propionate (prepared as described in DE 2,555,290) in 3 mL anhydrous tetrahydrofuran. Next, after holding for 1.5 hr at -50°C, the reaction was terminated by means of the addition of a saturated ammonium chloride solution. The resultant mixture was

then extracted with 2 x 50 mL ethyl acetate. The reunited organic phases were then rinsed with water, subjected to anhydrification over sodium sulfate, and evaporated under a desiccating vacuum. The obtained crude product material was then purified through flash chromatography, (ethyl acetate-3.8 *N* solution of ammonia in methanol 99:1). Finally, the recovered fraction was evaporated thoroughly, yielding 0.11 g of Compound 14 (yield rate: 15%).

^{1}H-NMR ($CDCl_3$, δ): 8.56 (dd, 2H, pyridine H6), 7.79 (dd, 2H, pyridine H3), 7.64 (ddd, 2H, pyridine H4), 7.10 (ddd, 2H, pyridine H5), 6.85 - 7.03 (m, 4H, methoxyphenyl aromatics), 3.84 (s, 3H, OCH_3), 2.95 - 3.12 (m, 4H, piperazine protons), 2.76 (t, 2H; $C(OH)C\underline{H_2}CH_2$), 2.55 - 2.75 (m, 4H, piperazine protons), 2.50 (t, 2H, $C(OH)CH_2C\underline{H_2}$).

Activity of Invention Compounds

The activity of the compounds specified in the present invention as inhibitors of frequency of micturition, as well as increasers of bladder capacity, renders them effective in the therapeutic treatment of neuromuscular dysfunctions of the lower urinary tract in mammals, including, among others, dysuria, urinary incontinence and enuresis.

The pharmacological activity of the invention compounds, in regard to inhibition of the frequency of urination and increasing of bladder capacity, render them well-suited for use in the therapeutic treatment of neuromuscular dysfunctions of the lower urinary tract, (including, for instance, dysuria, urinary incontinence and enuresis) in mammals.

The characteristics of the invention compounds render them significantly more potent in their anti-incontinence effect than flavoxate and imipramine, while they also possess an activity range which is more diverse than that of oxybutynin. These statements are supported by experimental data collected here from rat models, in trials which involved the inducing of rhythmic emptying contractions in rat bladders through their filling with a physiologic solution. The experimental compounds and a reference standard were evaluated in regard to their respective effects upon the frequency and amplitude of the bladder contractions, with particular attention being paid to the time interval, following administration of a given sample compound, during which the rhythmic bladder contractions disappeared.

Up to the present time, the therapeutic treatment of neuromuscular dysfunction of the lower urinary tract has involved the administration of compounds such as flavoxate, are active antispasmolytic drug which exerts a direct effect upon the musculature of the bladder, and, in addition, exerts an effect upon the pontine micturition center, anticholinergic compounds such as oxybutynin, and drugs with an action of a mixed type, such as imipramine (Andersson K. E., *Drugs of Today*, **24**(5), 337-348 (1988)).

However, the therapeutic agents which exert a direct effect upon the pelvic musculature (including the detrusor), may also produce undesirable side effects, such as incomplete bladder emptying, paralysis of the accommodation, tachycardia and dryness of the mouth (Andersson, *Drugs of Today*, **35**, 447 (1988)); while drugs of the imipramine type can display various toxic side effects, the most serious of which involve the cardiovascular system (orthostatic hypotension, ventricular arrhythmias), even at standard therapeutic doses.

On the basis of the discussion above, it is clearly desirable to increase the number of drugs available to the physician for the treatment of neuromuscular dysfunction of the lower urinary tract. The experimental data obtained here (and provided, below, in Table 1) includes a comparative evaluation of the effectiveness of the most widespread currently available drugs of this general type (flavoxate, oxybutynin and imipramine).

It was found here that the invention compounds exerted a significantly longer duration of activity (in particular, in regard to the maintaining of the rat bladder in a quiescent, non-contracting state represented by ED_{10min} in Table 1) than flavoxate, imipramine and oxybutynin. In addition, contrary to oxybutynin, the invention compounds were found to produce no significant reduction in the amplitude of the bladder contractions, indicating clearly that the action of the invention compounds does not compromise the contractile ability of the bladder.

Finally, the presence, in the invention compounds, of a heightened affinity for the type 5-HT_{1A} serotoninergic receptor (as indicated in the experimental data supplied below, in Table 2) indicates clearly that this receptor type plays a major role in the activity of the invention compounds. A detailed description of the various pharmacological tests conducted here, and the tables containing the collected experimental data, are provided below, in the section entitled "Pharmacological Data".

Therapeutic Applications

Patients who require treatment with medications of the type in question here are those who are suffering from neuromuscular dysfunctions of the lower urinary tract (as described by E. McGuire, in "Campbell's UROLOGY", 5th ed, W.B. Saunders Co., 616-638, 1986) as well as from various types of dysfunction associated with the compromising of the functionality of the type 5-HT_{1A} serotoninergic receptors.

The present invention also includes pharmaceutical formulations which contain the invention compounds described above, and, in addition, methods for the therapeutic use of such formulations in the treatment of neuromuscular dysfunctions of the lower urinary tract, such as dysuria, urinary incontinence and enuresis. Dysuria affects urinary frequency, nicturia and urine propulsion. The urinary incontinence syndrome includes force incontinence, urgency incontinence and emptying incontinence. Enuresis refers to the involuntary passage of urine, either at night or during the day.

Without desiring to be bound to a theory, we feel that it is important to mention that, in experimental studies, it has been found that the administration of type 5-HT_{1A} receptor antagonists prevents the undesirable activity of the sacral reflex arc and/or of the cortical mechanism which regulates micturition. This strongly suggests that the compounds specified in the present invention should be useful in the treatment of a wide range of types of neuromuscular dysfunction of the lower urinary tract.

The "effective quantity" of a compound which is employed in the treatment of urinary disturbances is considered to be that quantity of said compound which can produce a marked improvement of at least one symptom or parameter of such a disturbance.

Symptoms which are typically associated with functional disturbances of the urinary tract include urgency and frequency of urination, incontinence, loss of urine, enuresis, dysuria, difficult urination, and difficulty in completely evacuating the bladder.

An additional parameter in such disturbances is urine volume. The effective quantity of a pharmacological compound in the treatment of functional disturbances of the urinary tract can be established experimentally, with the corresponding development of a dosage quantity and frequency regimen, either by a single experimental team, or else by several teams working in close contact with one another. The exact quantity of medication which is

appropriate for administration to a given patient is contingent upon such factors as the state and severity of the complaint, the physical condition of the patient, and degree of observed (by an experienced physician) or patient-reported improvement of symptoms and parameters. It is understood that the clinically or statistically significant attenuation of symptoms and parameters of the conditions in question here is included within the scope of the present invention. In this context, the phrase clinically significant attenuation indicates a significant improvement, which is perceptible to the patient and/or to the physician.

The compounds of the present invention may be formulated in the form of a pharmaceutical liquid with a physiologically acceptable vehicle such as, for example, a physiologic solution prepared with phosphate or de-ionized water. The pharmaceutical formulation may also contain excipients, such as preservatives and stabilizers, whose pharmaceutical use has been well established. The invention compounds may be formulated as an orally-administered solid, a pill, capsule or powder, or in suppository form, and may include, with no specific limitations, such excipients as lubricants, plasticizers, coloration agents, absorption promoters, bactericides, etc.

The various modes of administration for these formulations of the present invention include oral, parenteral, intravenous, intramuscular, subcutaneous, transdermal, transmucosal (oral and rectal tablets) and by inhalation. Either oral or transdermal administration of the invention compounds is preferred (in the form, respectively, of an oral solid or liquid, and cutaneous porous plasters.

The quantity of the therapeutic agent in question which is to be administered varies from ca. 0.01 mg/kg/day to ca. 25 mg/kg/day, with a preferred dosage range of from ca. 0.1 mg/kg/day to 10 mg/kg/day, and with an optimal dosage range of 0.2 - 5 mg/kg/day. It is understood that the pharmaceutical formulation of the present invention is not necessarily required to contain the entire quantity of active agent for the treatment of the urinary disturbance, since such an effective-quantity may also be attained through the administration of a number of doses of the same pharmaceutical formulation.

In one of the preferred versions of the present invention, the invention compounds are formulated in pill or capsule form, each of which contains, by preference from 50 mg to 200 mg of the pharmaceutically active invention compounds). It is highly recommended,

also, that the daily dosage administered to the patient fall within the general range of 50 - 400 mg, and, by preference, 150-250 mg, with an ideal daily dosage level of 200 mg. This is the recommended regimen for use in the effective treatment of urinary incontinence and dysfunctions associated with the compromising of the functionality of the 5-HT_{1A} receptors.

The Examples, methodological descriptions and data tables provided here supply a very comprehensive illustration of the preferred version of present invention, and demonstrate its applicability as well as the advantages of its use. However, the potential application of this invention is not intended to be limited to these Examples, descriptions and experimental data.

Pharmacological Data

Effects on rhythmic bladder-voiding contractions induced by bladder filling in anaesthetised rats

A. *Methods:*

Female Sprague Dawley rats weighing 225 - 275 g (Crl: CDo BR, Charles River, Italy) were employed. Each animal had free access to food and water and was exposed to an alternating cycle of 12 hr light / 12 hr darkness, at a temperature of 22-24°C, for at least 1 week, except while the experiment was being conducted. Rhythmic contractile activity for bladder emptying was evaluated here in accordance with the method provided by Dray (J. Pharmacol. Methods, 13:157, 1985), with certain modifications, as described by Guarneri (Pharmacol. Res., 27:173, 1993). In brief, the rats were anaesthetized with a subcutaneous injection of 1.25 g/kg (5 ml/kg) urethane, after which the bladder was catheterized through the urethra, using a PE 50 polyethylene tube filled with a physiologic solution. The catheter was fixed in position around the external urethral orifice with a ligature, and was connected to a standard pressure transducer (Statham P23 ID/P23 XL). The pressure level within the bladder was continuously displayed on a band-type recorder unit (Battaglia Rangoni KV 135, equipped with a DC1/TI amplifier). The bladder was supplied with incremental doses of the physiologic filling solution, through the registration catheter, until the appearance of

reflex emptying contraction of the bladder (in general, after the reception by the bladder of 0.8 - 1.5 ml of the filling solution). For the intravenous (i.v.) injection of the experimental compounds, a PE 50 polyethylene tube filled with a physiologic solution was inserted into the jugular vein.

Cystometrograms were obtained, from which were derived the number of bladder contractions registered 15 min before (basal value) and after the treatment procedure, as well as mean contraction amplitude (average peak height, in mmHg).

Since most of the experimental compounds studied here produce their effect relatively quickly, and cause the complete cessation of bladder contraction, respective bioactivity levels were easy to assess, by means of the measurement of the duration of the period of bladder quiescence (that is, the time interval during which there were no bladder contractions). The number of experimental animals exhibiting a reduction in number of bladder contractions was also recorded here, when there was noted a contraction level of 30% or less, in relation to the number of contractions observed during the basal period.

In order to compare the potencies of the various experimental compounds in regard to the inhibition of bladder emptying contractions, values were calculated here (through the use of linear regression analysis with the least square method) for equi-effective dosage levels, which produced 10 min of bladder quiescence (disappearance of bladder contractions); these values are provided below, in Table l, under the heading ED_{10} min Dosage values were also extrapolated here (through the use of the method provided by Bliss (Bliss C.I., Quart. J. Pharm. Pharmacol., 11, 192-216, 1938), which produced a reduction in contraction frequency to 30% or less of basal value in at least 50% of the treated rats; this value is represented in Table 2 in the column headed ED_{50} (frequency). After the cessation of the effect produced by the injection of each experimental drug, values for peak height were obtained; these values were then compared with those previously obtained following the intravenous administration of the vehicle alone, with no active pharmacological agent. This provided a basis for the evaluation of the respective potencies of the various experimental substances on the amplitude of bladder contraction again, through the use of the method provided by Bliss (Bliss C.I., Quart. J. Pharm. Pharmacol., 11, 192-216, 1938); the obtained extrapolated ED_{50} (amplitude) values, supplied below, in Table 1, represents a

reduction of at least 30% in the amplitude of bladder contraction in at least 50% of the treated rats.

B. *Results*

The rapid distension (filling) of the bladder of a rat previously anaesthetized with urethane generates a series of rhythmic bladder emptying contractions, which have been described in the following articles: Maggi et al, Brain Res., 380:83, 1986; Maggi et al, J. Pharmacol. Exper. Ther., 230:500, 1984. The frequency of these rhythmic contractions is associated with the sensitive afferent branch of the micturition reflex, and with the integrity of the micturition center, while contraction amplitude is a function of the efferent branch of the same reflex arc. In this model, compounds (such as morphine) which act primarily upon the central nervous system, cause a blockage of the emptying contractions of the bladder, while other drugs (such as oxibutinin) which act at the level of the detrusor, reduce the amplitude of bladder contractions.

Results obtained for the various experimental compounds evaluated here are provided, below, in Table 1.

TABLE 1

Effects on rhythmic bladder-voiding contractions induced by bladder filling upon intravenous administration

Compound	$ED_{10\ min}$ µg/kg	ED_{50} (frequency) µg/kg	ED_{50} (amplitude) µg/kg
Example 5	523	77	n.a.
Example 6	225	93	n.a.
Example 7	78	18	n.a.
Example 8	74	2.5	n.a.
Example 9	77	25	n.a.
Example 10	228	180	n.a.
Flavoxate	> 10,000	2,648	n.a.
Oxybutynin	7,770	10,000	240
Imipramine	> 6,000	1,676	2,930

(n.a. = non-active; no significant reduction of peak heights.]

The values provided here are defined as follows: $ED_{10\ min}$ - the extrapolated value for the dosage of a test material which is required to induce 10 min of bladder quiescence; ED_{50} (frequency) - the extrapolated value for the dosage of a test material required to reduce contractions to less than 30% of the established basal level in at least 50% of the treated rats; and ED_{50} (amplitude) - the extrapolated value for the dosage of a test material required to reduce contraction amplitude by at least 30% from the established basal level, in at least 50% of the treated rats.

All of the compounds of the present invention which were evaluated here exhibited a markedly higher level of effectiveness in the inhibition of emptying contractions of the bladder (as reflected in the obtained values for $ED_{10\ min}$ or ED_{50}) than did flavoxate, imipramine and oxybutynin. In addition, contrary to flavoxate, oxybutynin and imipramine, the invention compounds evaluated here did not exert a significant effect upon bladder contractility capacity, indicating that there was no compromising of bladder contractility potential in these cases.

Binding affinities of the invention compounds for 5-HT_{1A} receptors, and other receptor types

A. *Methods*

Human Recombinant 5-HT_{1A} Receptors

The genomic codifier of the G-21 clone of the human 5-HT_{1A} serotoninergic receptor was first transferred, in a stable manner, into a human cell line (HeLa). The HeLa cells were then cultured in monolayers, in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf and 100 mg/ml gentamicin, under 5% CO_2 at 37°C. The cells were subsequently removed from the culture flask, at 95% confluence, with the aid of a cellular collector device, and was then subjected to lysis in ice cold 5 mM Tris buffer and 5 mM EDTA (pH 7.4). The obtained homogenates were then subjected to centrifuging at 40,000 x g, for a period of 20 min, and the membrane was then resuspended in a small volume of ice cold 5 mM Tris buffer and 5 mM EDTA (pH 7.4), followed by immediate freezing and preservation until the moment of use. The day of the experiment, the cellular

membrane was resuspended in a binding buffer which consisted of the following: 50 mM tris-HCl (pH 7.4); 2.5 mM $MgCl_2$; 10 μM pargyline (Fargin et al, Nature 355,358-360, 1988). The membrane was then incubated with [^{3}H]8-OH-DPAT 0.2 - 1 nm, in a final volume of 1 mL, for 30 min, at a temperature of 30°C, in the presence or the absence of the experimental substance. The non-specific binding was found to be stable in the presence of 5-HT 10 μM. Incubation was arrested through the addition of ice cold Tris-HCl buffer; was followed by rapid filtration through either Whatman GFB filters or Schleicher & Schuell GF52 filters, pre-treated with 0.2% polyethyleneamine.

Native 5-HT_{2A} Serotoninergic Receptors (Animal Tissue)

The studies of binding to type 5-HT_{2A} serotoninergic native receptors (Craig A. and Kenneth J., Life Sci. 38, 117-127, 1986)) were carried out here through the use of the membrane of the rat cerebral cortex. Cervical luxation was first carrried out on male Sprague Dawley rats (200-300 g, SD Harlan/Nossan, Italy); the brains were dried, followed by immediate freezing and preservation, at a temperature of -70°C, until the moment the cerebral cortex was required for experimental use. The thawed sample tissues were homogenated (2 x 20 sec) in 50 volumes of cold 50 mM Tris-HCl buffer (pH 7.4), through the use of a Politron homogenation unit (at speed setting 7). The obtained homogenates were then subjected to centrifuging at 49,000 x g, for a period of 10 min, and were then resuspended in 50 volumes of the same buffer, incubated for 15 min at 37°C, then centrifuged and re-suspended two more times. The processed membrane was then suspended in 100 volumes of 50 mM Tris-HCl (pH 7.7). The processed membrane was then incubated in a final volume of 1 ml, for 20 min, at a temperature of 37°C, with 0.7 - 1.3 nM [^{3}H]Ketanserin, in the presence or the absence of competitor substances. The non-specific binding was determined in the presence of 2 μM ketanserin. Incubation was then halted through the addition of ice cold 50 mM Tris-HCl buffer; this was followed by rapid filtration through Whatman GFB or Schleicher & Schuell GF52 filters, which were pre-treated with 0.2% polyethyleneamine. The filters were then rinsed with ice cold buffer, and the amount of radioactivity retained by the filters was evaluated through the use of liquid scintillation spectrometry.

Native α_1-Adrenergic Receptors (Animal Tissue)

The studies of binding to type α_1-adrenergic native receptors were carried out here through the use of the membrane of the rat cerebral cortex. Cervical dislocation was first carrried out on male Sprague Dawley rats (200-300 g, Charles River, Italy); the brains were dried, followed by immediate freezing and preservation, at a temperature of -70°C, until the moment the cerebral cortex was required for experimental use. The thawed sample tissues were homogenated (2 x 20 sec) in 50 volumes of cold 50 mM Tris-HCl buffer (pH 7.4), through the use of a Politron homogenation unit (at speed setting 7). The obtained homogenates were then subjected to centrifuging at 48,000 x g, for a period of 10 min, and were then resuspended in 50 volumes of the same buffer, incubated for 15 min at 37°C, then centrifuged and re-suspended two more times. The processed membrane was then suspended in 100 volumes of 50 mM Tris-HCl buffer (pH 7.4), which contained 10 μM pargyline and 0.1% ascorbic acid. The processed membrane was then incubated in a final volume of 1 ml, for 30 min, at a temperature of 25°C, with 0.5 - 1.5 nM [^{3}H]prazosin, in the presence or the absence of the sample substance. The non-specific binding was determined in the presence of 10 μM phentolamine. Incubation was then halted through the addition of ice cold 50 mM Tris-HCl buffer; this was followed by rapid filtration through Whatman GFB or Schleicher & Schuell GF52 filters, which were pre-treated with 0.2% polyethyleneamine. The filters were then rinsed with ice cold buffer, and the amount of radioactivity retained by the filters was evaluated through the use of liquid scintillation spectrometry.

Analysis of Obtained Data

Calculations were made here for IC_{50} values, as a means of evaluating the degree of inhibition of the specific bond of the radioligands, on the part of the investigated compounds, through the use of the Allfit non-linear interpolation program (De Lean et al, Am. J. Physiol., 235, E97 - E102, 1978). Obtained IC_{50} values were converted to values for affinity constant (Ki), through the use of the equation of Cheng & Prusoff (Cheng Y.C., Prusoff W.H., Biochem. Pharmacol. 22, 3099 - 3108, 1973).

B. *Results*

The results provided below, in Table 2, indicate that the invention compounds possess a strong affinity for type 5-HT_{1A} serotoninergic receptors, and a much weaker affinity for types 5-HT_{2A} and α_1-adrenergic receptors.

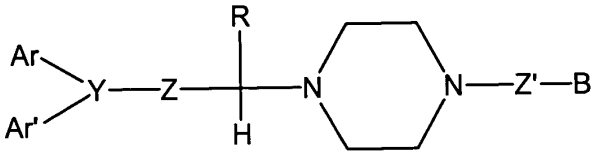
TABLE 2

Relative bonding affinities of the invention compounds for 5-HT_{1A} receptors, and other receptor types (all data expressed in Ki (nm))

Compound	5-HT_{1A}	5-HT_{2A}	α_1
Example 1	3.9	320	145
Example 2	0.6	159	208
Example 7	7.7	140	396
Example 8	3.97	320	191
Example 9	0.62	1,023	268
Example 10	19.3	683	1,322
Example 12	1.45	---	226
Example 14	0.34	---	114

CLAIMS

1. Compounds of general formula I



(I)

where

both Ar and Ar' represent, separately, either a substituted or nonsubstituted aryl group, or a substituted or nonsubstituted heteroaryl group,

Y represents a nitrogen atom or a CH, C-OH, C-CN or C-$CONH_2$ group,

R represents a hydrogen atom, or a lower alkyl group,

B represents a substituted or unsubstituted alkyl or heteroaryl group,

Z represents a methylene or ethylene group, and

Z' represents a valence bond or a methylene or ethylene group,

and enantiomers, diastereoisomers, N-oxides, polymorphs, solvates and pharmaceutically acceptable salts of such compounds.

2. Any of the following compounds:

1-(3,3-diphenylpropyl)-4-(2-methoxyphenyl)piperazine,

1-(3,3-diphenylpropyl)-4-[5-(2,3-dihydro-1,4-benzodioxinyl)]piperazine,

1-[3,3-*bis*-(4-nitrophenyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[3,3-*bis*-(4-methoxyphenyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[*N,N-bis*-(2-pyridyl)-2-aminoethyl]-4-(2-methoxyphenyl)piperazine,

1-[3-cyano-3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[3-cyano-3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[3-carbamoyl-3-phenyl-3-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine,

1-[*N*-(2-nitrophenyl)-*N*-(2-pyridyl)-2-aminoethyl]-4-(2-methoxyphenyl)piperazine,

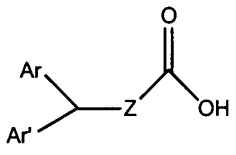
1-[3-cyano-3-(2-nitrophenyl)-3-phenylpropyl]-4-(2-methoxyphenyl)piperazine,

1-[3-carbamoyl-3-(2-nitrophenyl)-3-phenylpropyl]-4-(2-methoxyphenyl)piperazine, and 1-[3-hydroxy-3,3-*bis*-(2-pyridyl)propyl]-4-(2-methoxyphenyl)piperazine.

3. Pharmaceutical compositions which contain compounds formula I, as defined in claim 1, or the pharmaceutically acceptable diastereoisomers, enantiomers, *N*-oxides, polymorphs, solvates or salts of such compounds, in mixture with certain pharmaceutically acceptable diluents or vehicles.

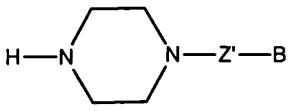
4. The use of compounds of Claim 3, in the treatment of patients suffering from neuromuscular dysfunctions of the lower urinary tract, said treatment regimen involving the administration to such patients of a therapeutically effective quantity of compounds of general formula I, which are represented in Claim 1, or the pharmaceutically acceptable diastereoisomers, enantiomers, N-oxides, polymorphs, solvates or salts of such compounds.

5. A process for the preparation of compounds of formula I wherein Ar, Ar', B, Z and Z' are defined as above in Claim 1, Y reprsents a CH group, and R represents a hydrogen atom, said process involving the reacting a compound II



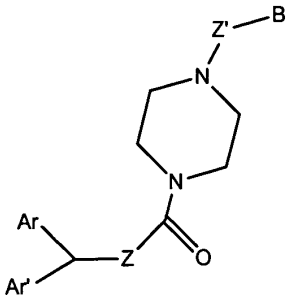
II

wherein Ar, Ar' and Z have the same meanings as in Claim 1, with a piperazine derivative of formula



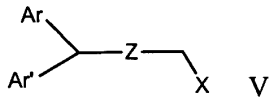
where Z' and B are defined as in Claim l,

and reduction of the obtained compound III



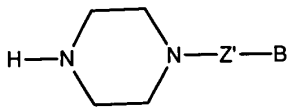
III .

6. A process for the preparation of compounds of formula I where Ar, Ar', B, Z and Z' are defined as in Claim 1, Y represents a CH group, and R represents a hydrogen atom, said process involving the reacting of a compound of formula V



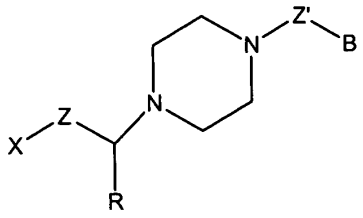
V

where Ar, Ar' and Z are defined as above in Claim 1 and X represents a leaving group such as, a halogen atom, or an alkylsulfonyloxy or arylsulfonyloxy group, with a piperazine derivative of formula



where Z' and B are defined as above in Claim 1.

7. A process for the preparation of compounds of formula I where Ar, Ar', R, B, Z and Z' are defined as above in Claim 1, and Y represents a CH group, said process involving the alkylation of the carbanion of a compound $ArCH_2Ar'$ where Ar and Ar' are defined as in Claim 1, with a compound of formula VI

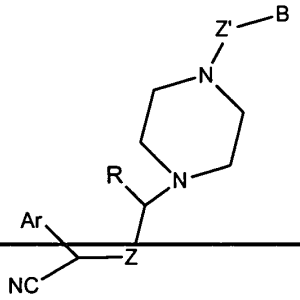


VI

where R, B, Z and Z' are defined as in Claim 1, and X is defined as above in Claim 6.

8. A process for preparation of compounds of formula I, where Ar, Ar', R, B, Z and Z' are defined as in Claim 1, and Y represents a C-CN group, said procedure involving the alkylation of the carbanion of a compound ArCH(CN)Ar' where Ar and Ar' are defined as in Claim 1, with a compound of formula VI as defined above in Claim 7.

9. A process for preparation of compounds of formula I, where Ar, Ar', R, B, Z and Z' are defined as above in Claim 1, and Y represents a C-CN group, said process involving the reacting of a compound $ArCH_2CN$ with a compound of formula VI as defined in Claim 7, followed by the arylation of the obtained compound IX



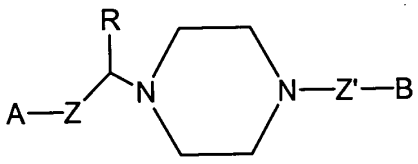
IX

with an Ar'-Hal compound, whre Ar' is defined as in Claim 1, and Hal represents a halogen atom.

10. A process for the preparation of compounds of formula I, where Ar, Ar', R, B, Z and Z' are defined as in Claim 1, and Y represents a C-$CONH_2$ group, said process involving the hydrolysis of a corresponding compound I, in which Y represents a C-CN group, with an aqueous acid or a Lewis acid, at a maximum temperature of 80°C.

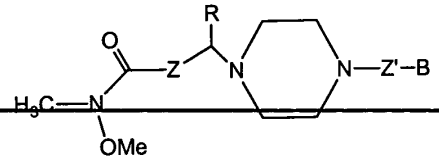
11. A process for the preparation of compounds of formula I, where Ar, Ar, R, B, Z and Z' are defined as in Claim 1, and Y represents a CH group, said process involving the hydrolysis of a corresponding compound I in which Y represents a C-CN or C-$CONH_2$ group, with at least 70% sulfuric acid, under reflux conditions.

12. A process for the preparation of compounds of structural formula I, where Ar, Ar', R, B, Z and Z' are defined as in Claim 1, Ar' is the same as Ar and Y represents a C-OH group, said process involving the reacting of a Ar-Met metallic aryl derivative in which the term Ar is defined as in Claim 1 and Met represents a metal such as lithium or magnesium with a compound X where R, B, Z and Z' are defined as in Claim 1 and A represents a carboxylate, cyano, or carbamoyl group.



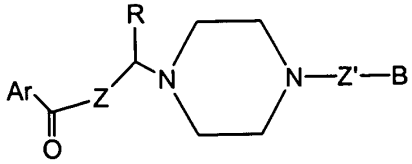
X

13. A process for the preparation of compounds of formula I where Ar, Ar', R, B, Z and Z' are defined as in Claim 1 and Y represents a C-OH group, said process involving the reacting of an aryl metallic derivative Ar-Met with a compound X'



X'

where R, B, Z and Z' are defined as in Claim 1, and the subsequent reaction of the obtained compound XI

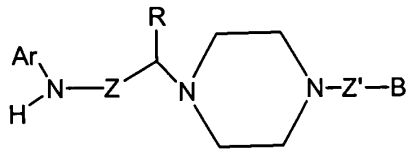


XI

with an Ar'-Met metallic aryl derivative where Ar' is defined as in Claim 1, and Met is as defined in the present Claim.

14. A process for the preparation of compounds of formula I where Ar, Ar', R, B, Z and Z' are defined as above, in Claim 1 and Y represents a nitrogen atom, said process involving the reacting of a the aza-anion of ArHNAr' compound in Ar and Ar' are defined as in Claim 1, with a compound VI as represented in Claim 7.

15. A process for the preparation of compounds of formula I, where Ar, Ar', R, B, Z and Z are defined as in Claim 1, and Y represents a nitrogen atom, said procedure involving the reacting of a compound Ar'-X where Ar' is defined as in Claim 1 with a compound XIII



XIII

where Ar, R, B, Z and Z' are defined as in Claim 1.

16. The use of pharmaceutical compositions of the types described in Claim 3, for the treatment of patients suffering from disturbances of the central nervous system, such as anxiety and depression, hypertension, disturbances of the sleeping/waking cycle, alimentary action and/or sexual function, and cognitive disturbances, this treatment regimen involving administration, to the patient, of a therapeutically effective quantity of compounds of formula I as defined in Claim l, or the enantiomers, diastereoisomers, N-oxides, polymorphs, solvates or pharmaceutically acceptable salts of such compounds.

*****

Milan, August 1, 1997

RF 13

MODULARIO
I.C.A. - 101

Mod. C.E. - 1-4-7

MINISTERO DELL'INDUSTRIA, DEL COMMERCIO E DELL'ARTIGIANATO

DIREZIONE GENERALE DELLA PRODUZIONE INDUSTRIALE
UFFICIO ITALIANO BREVETTI E MARCHI

Autenticazione di copia di documenti relativi alla domanda di brevetto per INV. IND.

N. MI97 A 001861

Si dichiara che l'unita copia è conforme ai documenti originali depositati con la domanda di brevetto sopraspecificata, i cui dati risultano dall'accluso processo verbale di deposito

Roma, lì 23 LUG 1998

IL DIRETTORE DELLA DIVISIONE
IL PRIMO DIRIGENTE
(Ing. Attilio Ronzacci)

MO:

AL MINISTERO DELL'INDUSTRIA DEL COMMERCIO E DELL'ARTIGIANATO
UFFICIO ITALIANO BREVETTI E MARCHI - ROMA

DOMANDA DI BREVETTO PER INVENZIONE INDUSTRIALE, DEPOSITO RISERVE, ANTICIPATA ACCESSIBILITÀ AL PUBBL

A. RICHIEDENTE (I)

1) Denominazione: RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A. — N.G. — SP

Residenza: MILANO – ITALIA — codice 00743210150

2) Denominazione:

Residenza: — codice

B. RAPPRESENTANTE DEL RICHIEDENTE PRESSO L'U.I.B.M.

cognome nome — cod. fiscale

denominazione studio di appartenenza

via — n. — città — cap — (prov)

C. DOMICILIO ELETTIVO destinatario: RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A.

via Matteo Civitali n. 01 città MILANO cap 20148 (prov) MI

D. TITOLO classe proposta (sez/cl/scl) A61K gruppo/sottogruppo /

Diarilalchilpiperazine attive sulle basse vie urinarie

ANTICIPATA ACCESSIBILITÀ AL PUBBLICO: SI [ ] NO [X] SE ISTANZA: DATA / / Nº PROTOCOLLO

E. INVENTORI DESIGNATI (cognome nome)

1) LEONARDI Amedeo
2) MOTTA Gianni
3) RIVA Carlo
4) TESTA Rodolfo

F. PRIORITÀ

	nazione o organizzazione	tipo di priorità	numero di domanda	data di deposito	allegato S/R
1)				/ /	
2)				/ /	

SCIOGLIMENTO RISERVE — Data — Nº Protocollo

G. CENTRO ABILITATO DI RACCOLTA COLTURE DI MICRORGANISMI, denominazione

H. ANNOTAZIONI SPECIALI

DOCUMENTAZIONE ALLEGATA

	N. es.			
Doc. 1)	1	PROV	n. pag. 48	riassunto con disegno principale, descrizione e rivendicazioni (obbligatorio 1 esemplare) .....
Doc. 2)		PROV	n. tav.	disegno (obbligatorio se citato in descrizione, 1 esemplare .....
Doc. 3)		RIS		lettera d'incarico, procura o riferimento procura generale .....
Doc. 4)	1	RIS		designazione inventore .....
Doc. 5)		RIS		documenti di priorità con traduzione in italiano .....
Doc. 6)		RIS		autorizzazione o atto di cessione .....
Doc. 7)				nominativo completo del richiedente

SCIOGLIMENTO RISERVE — Data — Nº Protocollo

confronta singole priorità

3) attestati di versamento, totale lire 565.000 - cinquecentosessantacinque mila obbligatorio

COMPILATO IL 01/08/1997 **FIRMA DEL(I) RICHIEDENTE (I)** Abraham SARTANI - Direttore R&S

CONTINUA SI/NO NO RECORDATI INDUSTRIA CHIMICA E FARMACEUTICA S.p.A.

DEL PRESENTE ATTO SI RICHIEDE COPIA AUTENTICA SI/NO SI

UFFICIO PROVINCIALE IND. COMM. ART. DI MILANO codice 15

VERBALE DI DEPOSITO NUMERO DI DOMANDA MI97A 001861 Reg.A

L'anno millenovecento NOVANTASETTE il giorno UNO del mese di AGOSTO

il(i) richiedente(i) sopraindicato(i) ha(hanno) presentato a me sottoscritto la presente domanda, corredata di n. 00 fogli aggiuntivi per la concessione del brevetto sopraripportato.

I. ANNOTAZIONI VARIE DELL'UFFICIALE ROGANTE

IL DEPOSITANTE — timbro — L'UFFICIALE ROGANTE

PROSPETTO A

RIASSUNT INVENZI NE C N DISEGNO PRINCIPALE, DESCRIZIONE E RIVENDICAZIONE

NUMERO DOMANDA MI97A 001861 REG. A

NUMERO BREVETTO

DATA DI DEPOSITO 01/08/1997

DATA DI RILASCIO / /

D. TITOLO

"DIARILALCHILPIPERAZINE ATTIVE SULLE BASSE VIE URINARIE"

L. RIASSUNTO

Vengono descritti composti diarilalchilpiperazinici attivi sulle basse vie urinarie. I composti e i loro enantiomeri, diastereoisomeri, N-ossidi, polimorfi, solvati e sali farmaceuticamente accettabili sono efficaci nel trattamento di pazienti affetti da disfunzioni neuromuscolari delle basse vie urinarie e per il trattamento delle patologie associate al recettore $5\text{-}HT_{1A}$. Sono descritti anche la preparazione dei composti e le composizioni farmaceutiche che li contengono.

RECORDATI, Industria Chimica e Farmaceutica S.p.A.

M. DISEGNO

DESCRIZIONE dell'invenzione avente per titolo:

"DIARILALCHILPIPERAZINE ATTIVE SULLE BASSE VIE URINARIE"

a nome : Recordati, Industria Chimica e Farmaceutica S.p.A.

con sede in: Milano, via Civitali, 1

di nazionalita': italiana.

Inventori designati: Amedeo LEONARDI, Gianni MOTTA, Carlo RIVA e Rodolfo TESTA

*****

AMBITO DELL'INVENZIONE

L'invenzione riguarda diarilalchilpiperazine, le composizioni farmaceutiche che le contengono e gli usi di tali derivati e composizioni.

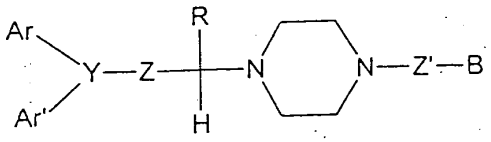
Il flavossato, l'ossibutinina e l'imipramina sono principi attivi rappresentativi di tre diverse classi di composti attualmente usate nella terapia dell'incontinenza urinaria. Tali sostanze sono state sperimentate in modelli animali nei quali è stata confermata la loro attivita'.

I composti dell'invenzione, riportati di seguito, condividono ~~poche caratteristiche strutturali con le suddette sostanze,~~ tranne la sola presenza nella molecola di un atomo di azoto basico.

I composti dell'invenzione dimostrano una maggiore efficacia rispetto ai suddetti farmaci nei test farmacologici indicativi dell'attivita' sulle basse vie urinarie, in particolare dell'attivita' contro le disfunzioni neuromuscolari delle basse vie urinarie, e sono dotati di un'affinita' potente e selettiva per il recettore 5-HT_{1A}

serotoninergico.

Sotto un certo aspetto, l'invenzione riguarda composti di formula I:



(I)

dove

sia Ar sia Ar' separatamente rappresentano un gruppo arile sostituito o non sostituito o un gruppo eteroarile sostituito o non sostituito,

Y rappresenta un atomo di azoto o un gruppo CH, C-OH, C-CN o $C-CONH_2$,

R rappresenta un atomo di idrogeno o un gruppo alchile inferiore,

B rappresenta un gruppo arile sostituito o non sostituito o un gruppo eteroarile sostituito o non sostituito,

Z rappresenta un gruppo metilene o etilene, e

Z' rappresenta un legame di valenza o un gruppo metilene o etilene.

L'invenzione riguarda anche gli enantiomeri, i diastereoisomeri, gli N-ossidi, i polimorfi, i solvatati e i sali farmaceuticamente accettabili di tali composti.

L'invenzione riguarda inoltre le composizioni farmaceutiche comprendenti i composti di formula I o gli enantiomeri, i diastereoisomeri, gli N-ossidi, i polimorfi, i solvatati e i sali farmaceuticamente accettabili in miscela con diluenti e veicoli

farmaceuticamente accettabili.

L'invenzione riguarda inoltre l'uso di tali composizioni per il trattamento dei pazienti affetti da disfunzioni neuromuscolari delle basse vie urinarie, in particolare mediante riduzione della frequenza delle contrazioni vescicali dovute alla distensione della vescica e all'aumento della capienza della vescica stessa, l'uso prevede la somministrazione al paziente di una quantita' terapeuticamente efficace di uno o piu' composti selezionati di formula I o degli enantiomeri, diastereoisomeri, N-ossidi, polimorfi, solvatati e sali farmaceuticamente accettabili dei composti stessi.

Secondo un ulteriore aspetto, l'invenzione riguarda metodi di interazione con i recettori serotoninergici 5-HT_{1A} e quindi, in virtu' dell'attivita' svolta a livello di questo recettore, il possibile impiego nel trattamento di disturbi del sistema nervoso centrale quali l'ansia e la depressione, l'ipertensione, i disturbi del ciclo sonno/veglia, il comportamento alimentare e/o la funzionalita' sessuale e i disturbi cognitivi nei mammiferi, in particolare nell'uomo.

DESCRIZIONE DETTAGLIATA DELL'INVENZIONE

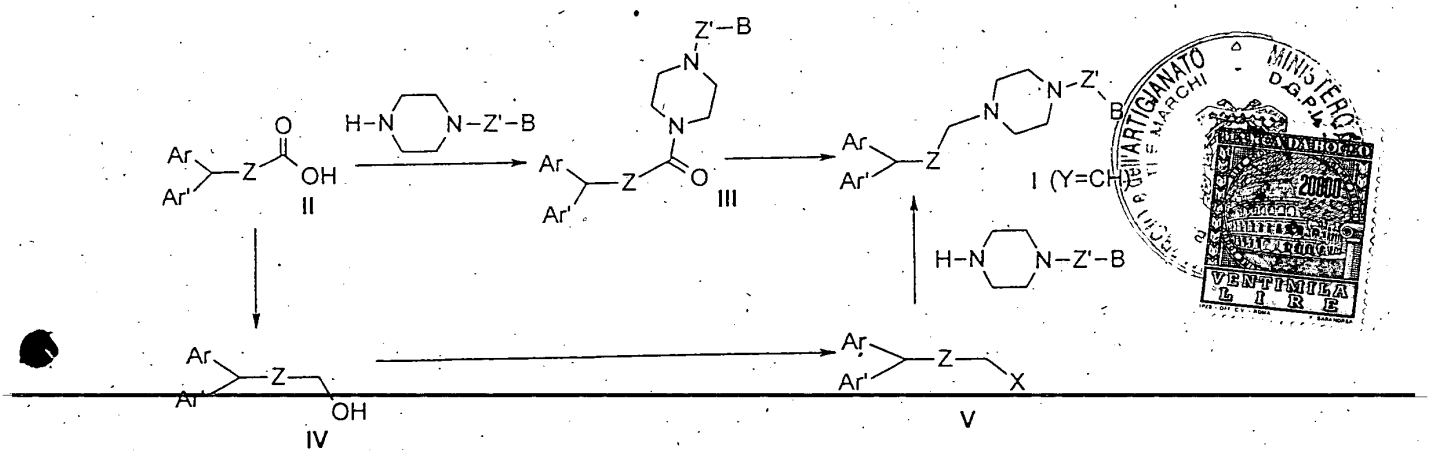
I gruppi arile che possono essere rappresentati da B, Ar e Ar' sono preferibilmente gruppi aromatici monociclici o biciclici con 6-12 atomi di carbonio (per esempio fenile o naftile). I gruppi eteroarile che possono essere rappresentati da B, Ar e Ar' sono preferibilmente gruppi aromatici monociclici o biciclici con 5-12 atomi di cui uno o piu' sono eteroatomi (per esempio azoto, ossigeno e zolfo) e i restanti sono atomi di carbonio. Uno o piu' sostituenti possono essere scelti

tra gli atomi di alogeno e i gruppi alchile, alcossi, alogenoalcossi, ciano, carbamoile, acile, nitro, ammino, acilammino, alchilsolfonil-ammino e alchilammino. Quando B rappresenta un gruppo arile, due sostituenti dell'anello aromatico possono essere collegati insieme per formare un'altro anello. Per esempio, B puo' rappresentare un anello benzodiossanile.

Sintesi dei composti dell'invenzione

I composti di formula I conformemente all'invenzione dove Y è un gruppo CH, R è H e Ar, Ar', B, Z e Z' sono come definiti sopra possono normalmente essere preparati come indicato nel seguente schema 1:

Schema 1



Gli intermedi di formula II sono reperibili in commercio oppure la loro sintesi è pubblicata nella letteratura e/o possono essere preparati con metodi tradizionali. In generale possono essere sintetizzati, per esempio per Z=metilene, dai corrispondenti diarilchetoni mediante reazione di Reformatsky con alchile

2-bromoacetato e zinco attivato (*Org. React.*, 1975, 22, 423; *Synthesis*, 1989, 571) seguita da idrolisi, o mediante reazione di Wadsworth-Emmons con trietil fosfonoacetato e base (*Chem. Rev.*, 1989, 89, 863) seguita da idrolisi. Ulteriori metodi di sintesi saranno ovvi o chi è esperto nell'arte.

Gli intermedi II possono essere condensati con idonei derivati piperazinici *N*-monosostituiti in presenza di un agente condensante (per esempio dietil cianosfosfonato, dicicloesilcarbodiimmide e N,N'-carbonildiimidazolo), eventualmente in presenza di un agente promotore (per esempio N-idrossisuccinimmide, 4-dimetilamminopiridina) in solvente aprotico o clorurato (per esempio N,N-dimetilformammide, cloroformio, metilene cloruro) a -20°C/140°C (Albertson, *Org. React.* 1962, *12*, 205-218: Doherty et al., *J. Med. Chem.* 1992, 35, 2; Staab et al., *NeWer Methods Prep. Org. Chem.*, 1968, 5, 61; Ishihara, *Chem. Pharm. Bull.* 1991, 39, 3236).

Altri tipi di reazione sono il metodo dell'anidride mista, con reazione dell'Intermedio II con un alchile cloroformiato in presenza di un'ammina terziaria (per esempio trietilammina) seguita dall'addizione del reagente piperazinico in solvente aprotico (per esempio diossano, metilene cloruro) eventualmente in presenza, per esempio, di 1-idrossipiperidina come agente promotore (*Org. React.* 1962, 12, 157).

Altri metodi di ammidificazione dell'Intermedio II (o derivati semplici di II quali gli esteri o gli acili cloruri) con piperazina N-monosostituita sono ovvi a chi è esperto nell'arte. Un ulteriore metodo di condensazione prevede la reazione di semplici alchil esteri

di II con le ammidi di alluminio ottenute dalle piperazine e dal trimetilalluminio (*J. Med. Chem.* 1996, 39, 4692).

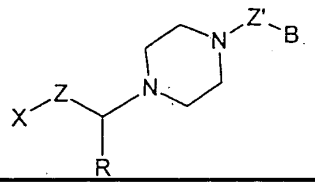
Gli Intermedi III cosi' preparati possono essere ridotti al composto (I) desiderato dove Y=CH ricorrendo ad agenti riducenti in grado di convertire la funzionalita' ammidica in funzionalita' amminica come l'idruro di litio alluminio o altri idruri complessi di alluminio in dietiletere o tetraidrofurano, o un complesso diboranico stabile come borano-tetraidrofurano o borano-dimetilsolfuro o altri (*J. Org. Chem.* 1982, 47, 1389) usati in solvente idoneo (per esempio tetraidrofurano). Questi composti del boro sono particolarmente efficaci quando i gruppi Ar portano gruppi riducibili come il nitro, che non vengono ridotti.

Altri agenti riducenti adatti sono noti a chi è esperto nell'arte (March, *Advanced Organic Chemistry*, Wiley Interscience Ed., 1992, 1212).

Una via di reazione alternativa per preparare i composti (I) dove Y è CH è di ridurre i composti II ricorrendo agli agenti riducenti suddetti o altre tecniche tradizionali (per esempio $NaBH_4$ con $CaCl_2$, ~~riduzione di anidridi miste ottenute per reazione con un cloroformiato,~~ con $NaBH_4$) in composti alcolici IV, che vengono convertiti nei reagenti alchilanti V, dove X è un gruppo uscente (Cl, I, Br, p-toluensolfonilossi, metansolfonilossi) per mezzo di tecniche tradizionali ben documentate. V puo' esser fatto reagire con piperazine monosostituite per dare i composti I. Tali reazioni di alchilazione sono eseguite con metodi tradizionali, ben noti a chi è esperto

nell'arte. In genere l'alchilazione è eseguita in solvente aprotico (per esempio acetonitrile, dimetilformammide, toluene, diossano, tetraidrofurano) o protico (per esempio etanolo, *n*-butanolo) o senza solvente in presenza o meno di una base (per esempio trietilammina, diisopropiletilammina, piridina, 4-dimetilamminopiridina, potassio carbonato) a temperatura compresa tra quella ambiente e 180 °C.

I composti IV e V possono essere preparati anche mediante alchilazione di un composto $ArCH_2Ar'$ nella forma del suo carbanione metilico (ottenuto per esempio con una base come butil litio o altro complesso di litio o altra base derivante da metallo alcalino) con un composto rispettivamente di formula $X-Z-CH_2-OPrG$ o $X-Z-CH_2-X$ dove X e Z sono come definiti sopra e PrG rappresenta un gruppo di protezione (per esempio O-tetraidropiranile) da rimuovere dopo l'alchilazione.
Se la stessa tecnica di alchilazione è eseguita su $ArCH_2Ar'$ con il seguente composto VI, dove X è un gruppo uscente come prima:



(VI)

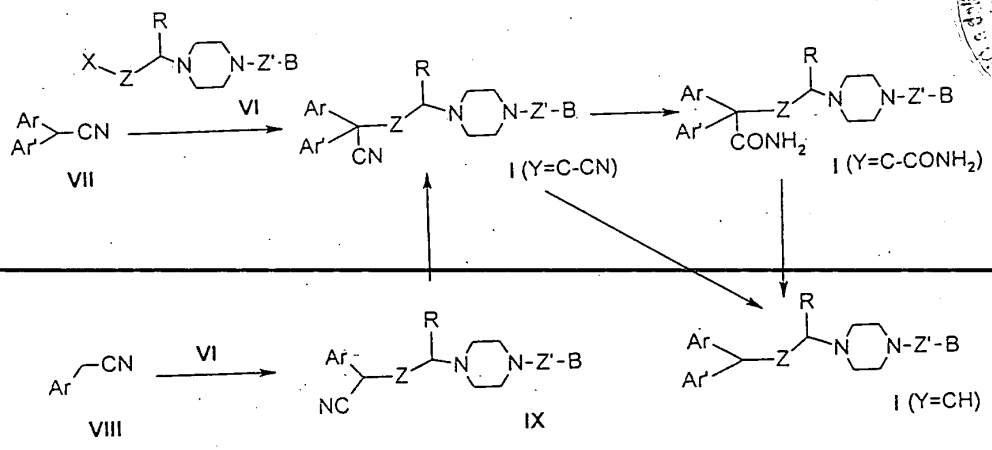
i composti I possono essere preparati direttamente.

I composti VI possono essere preparati facilmente partendo dai composti VI che hanno un gruppo COOAk invece del gruppo terminale $-CH_2-X$. Comuni procedimenti di riduzione (per esempio idruro di litio alluminio o altri idruri metallici complessi) possono dare i corrispondenti composti VI con X = OH, che possono a loro volta essere

tradizionalmente convertiti in composti VI con X = gruppo uscente. Gli esteri di partenza possono essere preparati con la ben nota reazione di Michael o reazioni di sostituzione nucleofila di una piperazina monosostituita su un idoneo estere 2,3-insaturo o 2-aloestere.

Tecniche alternative per ottenere i composti VI sono l'alchilazione di idonei derivati piperazinici monosostituiti con un composto rispettivamente di formula X-CH(R)-Z-OPrG o X-Z-CH_2-X dove X, R e Z sono come definiti sopra e PrG è un gruppo proteggente (per esempio O-tetraidropiranile) da rimuovere dopo l'alchilazione.
I composti di formula I conformemente all'invenzione dove Y è un gruppo C-CN, C-$CONH_2$ o CH e Ar, Ar', R, B, Z e Z' sono come definiti sopra possono generalmente essere preparati come indicato dal seguente Schema 2:

Schema 2



Gli Intermedi VII, normalmente reperibili in commercio o disponibili con metodi di sintesi tradizionali, possono essere trasformati in composti di formula I con Y = C-CN per alchilazione del

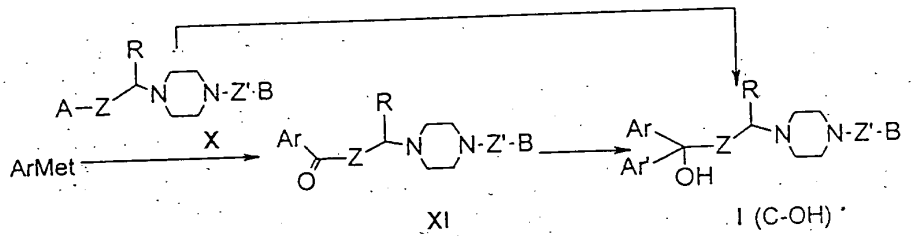
corrispondente carbanione con idonei derivati piperazinici VI (*Il Farmaco*, 1995, 50, 505). L'alchilazione è eseguita ricorrendo a una base (per esempio butil litio, sodio ammide, sodio idruro, litio diisopropilammide, litio *bis*(trimetilsilil)ammide o altra nota a chi è esperto nell'arte) in idoneo solvente aprotico come toluene, tetraidrofurano, dimetossietano, diossano, diglyme o altro) a -20°C/temperatura di ricaduta. I composti I dove Y = C-CN possono essere facilmente trasformati con tecniche tradizionali (idrolisi parziale con acido acquoso per esempio acido solforico al 70% o acido di Lewis normalmente a temperatura ambiente-80°C; March, *Advanced Organic Chemistry*, Wiley Interscience Ed., 1992, 887) in composti I con Y = C-$CONH_2$. L'idrolisi eseguita in condizioni piu' drastiche (per esempio acido solforico al 70% a temperatura di riflusso) consente un procedimento alternativo al metodo indicato nel primo schema di reazione dando composti I con Y = CH.

Un ulteriore tecnica consiste nell'eseguire la stessa C-alchilazione di cui sopra con idonei derivati piperazinici VI su composti $ArCH_2CN$ VIII, che sono normalmente disponibili in commercio o ~~accessibili con metodi di sintesi tradizionali, per dare gli intermedi~~ IX, che possono a loro volta essere arilati con un composto Ar'-LG (dove il gruppo uscente LG rappresenta un atomo di cloro, bromo o fluoro) normalmente eseguendo una reazione di trasferimento di fase in presenza di una base (per esempio sodio idrossido al 50%, *Tetrahedron Letter*, 1969 673) e un catalizzatore (per esempio trietilbenzilammonio cloruro) in idoneo solvente (per esempio toluene) a temperatura

ambiente/di ricadere. Il gruppo arile deve essere sufficientemente attivato alla sostituzione aromatica nucleofila dalla presenza di gruppi elettron-attrattori nella giusta posizione e/o in quanto eterociclo elettron-povero (*Chem. Rev.*, 1951, 49, 273).

I composti di formula I conformemente all'invenzione dove Y è un gruppo C-OH e Ar, Ar', R, B, Z e Z' sono come definiti sopra possono normalmente essere preparati come indicato nel seguente schema 3:

Schema 3

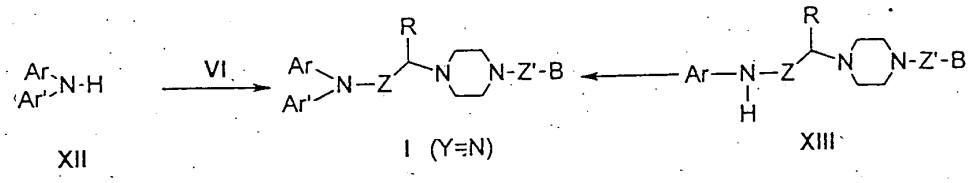


I derivati metallici arilici ArMet dove Met sta per metallo (per esempio litio o magnesio alogenuro) preparato facendo agire il butil litio o magnesio su un composto aril-Br o aril-I in tetraidrofurano o dietiletere a -70°C-temperatura di ricadere) sono fatti reagire nello stesso solvente a -20°C/temperatura di ricadere con derivati X facilmente preparabili con metodi tradizionali dove A può' rappresentare un gruppo carbossilato, ciano, $CONH_2$ per dare composti I dove Ar = Ar'. X è preferibilmente un alchile piperazinopropionato o un alchile piperazinoacetato. In alcuni casi è possibile la litiazione diretta della specie arile, per esempio nel caso in cui su Ar sia presente un sostituente *orto* dimetilamminocarbonile o metossi. Quando A in X rappresenta un gruppo $(CH_3O)(CH_3)NC(O)$ (A', ammide di Weinreb), è

possibile eseguire una reazione a due passaggi con isolamento degli intermedi chetonici XI. Gli XI sono poi fatti reagire con Ar'Met per dare i composti alcolici (I) anche portanti gruppi arile differenti.

I composti di formula I conformemente all'invenzione dove Y è un atomo di N possono normalmente essere preparati come indicato dal seguente schema 4:

Schema 4



XII I (Y=N) XIII

Gli intermedi XII possono essere trasformati per *N*-alchilazione del corrispondente aza-anione con idonei composti VI (vedi sopra). L'alchilazione è eseguita in presenza di una base (per esempio butil litio, sodio ammide, sodio idruro, litio diisopropilammide, litio bis(trimetilsilil)ammide o altra nota a chi è esperto nell'arte) in idoneo solvente aprotico come toluene, tetraidrofurano, dimetossietano, diossano, diglyme a -20°C/temperatura di ricadere.

Gli intermedi XII sono reperibili in commercio o possono essere preparati con procedimenti pubblicati nella letteratura in genere per sostituzione nucleofila di un composto Ar-NH_2 su un Ar'-LG (dove LG è un gruppo uscente come iodio, triflurometansolfonilossi, bromo, cloro, fluoro). La sostituzione nucleofila puo' essere catalizzata o non catalizzata ed è in genere eseguita in presenza di basi (per esempio sodio carbonato, litio diisopropilammide, sodio *tert*-butossido, ecc.).

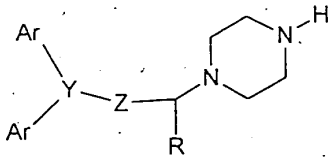
Il catalizzatore metallico usato puo' essere scelto tra un'ampia gamma, per esempio rame, rame (I) ioduro o bromuro o ossido (*Tetrahedron*, **1984**, 40, 1433), catalizzatori di nichel (*J. Org. Chem.*, **1975**, 40, 2267), palladio dicloruro, palladio diacetato, palladio tetrakis-(trifenilfosfino), bis(difenilfosfino)palladio dicloruro, palladio dibenzilidene acetone, bis(difenilfosfinoferrocene)palladio dicloruro (*Synlett*, **1996**, 329; *J. Org. Chem.*, **1997**, 62, 1568; **1997**, 62, 1268; **1997**, 62, 1264). Le reazioni sono eseguite a temperatura di fusione, senza solvente o in solvente adatto (per esempio dimetilacetammide, dimetilformammide, diossano, toluene, tetraidrofurano) a temperatura compresa tra quella ambiente e quella di ricadere in presenza o meno di un legante (per esempio trifenilfosfina, tri-o-tolilfosfina, bis(difenilfosfino)ferrocene, 2,2'-bis(difenilfosfino)-1,1'- binaftile o altro legante fosfinico reperibile in commercio).

Un procedimento alternativo per ottenere i composti (I), specialmente efficace quando uno o entrambi i gruppi arile portano gruppi nitro, consiste nell'arilare gli intermedi amminici XIII con la stessa tecnica riportata per la preparazione degli intermedi XII.

Gli intermedi XIII sono ottenibili con procedimenti comuni noti a chi è esperto nell'arte, che in genere consistono nell'alchilazione di un derivato anilinico Ar-NH_2 con un idoneo composto VI in solventi ad alto punto di ebollizione (per esempio *n*-butanolo) o a fusione. In alternativa, se la funzione arilica è adatta allo scopo (per esempio è sufficientemente attivata alla sostituzione aromatica nucleofila, vedi

sopra) possono essere preparati per reazione di un Ar-LG (dove LG è come definito sopra) con idoneo derivato omega-amminoalchil-piperazinico. La reazione puo' essere non catalizzata ed eseguita a temperatura di fusione senza solvente o in solvente idoneo (per esempio *n*-butanolo, dimetilformammide, dimetilacetammide) a temperatura ambiente/di ricadere. La reazione puo' essere catalizzata come per la preparazione degli intermedi XII.

Quando B è arile o eteroarile-alchilene inferiore, per preparare i composti I possono essere impiegate le suddette tecniche di reazione o, in alternativa, la sintesi puó' essere eseguita usando derivati piperazinici dove Z'-B rappresenta un gruppo proteggente (per esempio *tert*-butossicarbonile, benzilossicarbonile, benzile o altro opportunamente scelto tra gruppi proteggenti delle ammine in Greene, "Protective Groups in Organic Synthesis", Wiley Interscience, New York, 1991. Applicando gli stessi metodi generali di sintesi riportati sopra si ottengono i composti finali I dove Z'-B è il citato gruppo proteggente. Tecniche semplici e tradizionali di deprotezione consentono di preparare i composti XIV, che possono essere alchilati con idoneo arilalchile o eteroalchil alogenuro per dare detti composti I dell'invenzione.



XIV

ESEMPIO 1

1-(3,3-difenilpropil)-4-(2-metossifenil)piperazina cloridrato

a) 1-(3,3-difenilpropionil)-4-(2-metossifenil)piperazina (1A)

Ad una soluzione di 1,13 g di acido 3,3-difenilpropionico e di 1,06 g di 1-(2-metossifenil)piperazina in 25 mL di *N,N*-dimetilformammide, mantenuta in agitazione a 0-5°C, vennero aggiunti in successione 0,9 mL di dietil cianofosfonato 93% e 0,77 mL di trietilammina. La soluzione ottenuta fu agitata a temperatura ambiente per 5 h, versata in 250 mL di acqua ed estratta con etile acetato. La fase organica fu lavata con acqua, anidrificata su sodio solfato anidro ed evaporata sotto vuoto a secchezza. Il residuo oleoso fu purificato mediante cromatografia flash (cloroformio-etile acetato 9:1) a dare il prodotto del titolo con resa teorica.

^{1}H-NMR ($CDCl_3$, δ): 7,15-7,35 (m, 10H, protoni fenilici), 6,75-7,05 (m, 4H, protoni metossifenilici), 4,69 (t, 1H, CH), 3,85 (s, 3H, OCH_3), 3,67-3,77 (m, 2H, protoni piperazinici), 3,47-3,67 (m, 2H, protoni piperazinici), 3,10 (d, 2H, $CH_2C(O)$), 2,83-2,93 (m, 2H, protoni piperazinici), 2,67-2,77 (m, 2H, protoni piperazinici).

b) 1-(3,3-difenilpropil)-4-(2-metossifenil)piperazina cloridrato

~~Ad una soluzione di 2,0 g del composto 1A in 45 mL di~~ tetraidrofurano anidro mantenuta in agitazione a temperatura ambiente furono aggiunti a porzioni 0,44 g di litio alluminio idruro. La miscela di reazione fu agitata a temperatura ambiente per 24 h e per 2,5 h a riflusso. Successivamente la miscela fu raffreddata e furono aggiunti cautamente 5 mL di etile acetato e 5 mL di etanolo; quindi fu versata in 225 mL di acqua ed estratta con etile acetato. La fase organica fu

lavata con acqua, essiccata su sodio solfato anidro ed evaporata a secchezza sotto vuoto. Il grezzo fu purificato mediante cromatografia flash (etere di petrolio-etile acetato 7:3). Il residuo ottenuto per evaporazione delle frazioni raccolte fu sciolto in etile acetato ed alla soluzione fu aggiunto 1 equivalente molare di acido cloridrico (sol. 2N in etanolo). Il prodotto del titolo, cristallizzato, fu isolato mediante filtrazione a dare 0,83 g (39%). P.f. 143-149°C.

^{1}H-NMR ($CDCl_3$, δ): 12,75-13,10 (sa, 1H, NH+), 7,15-7,35 (m, 10H, protoni fenilici), 6,80-7,12 (m, 4H, protoni metossifenilici), 3,99 (t, 1H, CH), 3,85 (s, 3H, OCH_3), 3,38-3,70 (m, 6H, protoni piperazinici, CH_2NH+), 2,85-3,15 (m, 4H, protoni piperazinici), 2,65-2,82 (m, 2H, CH_2CH).

ESEMPIO 2

1-(3,3-difenilpropil)-4-[5-(2,3-diidro-1,4-benzodiossinil)]piperazina metansolfonato

a) 1-(3,3-difenilpropionil)-4-[5-(2,3-diidro-1,4-benzodiossinil)]-piperazina (2A)

Il prodotto del titolo fu preparato secondo la metodica descritta nell'esempio 1A, ma utilizzando la 1-[5-(2,3-diidro-1,4-benzo-diossinil)]piperazina invece della 1-(2-metossifenil)piperazina. Il grezzo fu purificato mediante cromatografia flash (cloroformio-etile acetato 8:2) a dare il prodotto del titolo (85%).

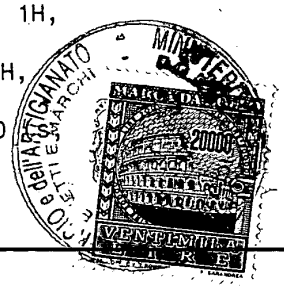
^{1}H-NMR ($CDCl_3$, δ): 7,15-7,35 (m, 10H, protoni fenilici), 6,74 (dd, 1H, H7 benzodiossanico), 6,60 (dd, 1H, H6 benzodiossanico), 6,40 (dd, 1H, H8 benzodiossanico), 4,68 (t, 1H, CH), 4,15-4,35 (m, 4H, OCH_2CH_2O),

3,65-3,75 (m, 2H,. protoni piperazinici), 3,40-3,50 (m, 2H, protoni piperazinici), 3,10 (d, 2H, $CH_2C(O)$), 2,85-2,95 (m, 2H, protoni piperazinici), 2,65-2,75 (m, 2H, protoni piperazinici).

b) 1-(3,3-difenilpropil)-4-[5-(2,3-diidro-1,4-benzodiossinil)]-piperazina metansolfonato

Il prodotto del titolo fu ottenuto e purificato seguendo la procedura riportata nell'esempio 1 utilizzando l'intermedio 2A invece di 1A. Il residuo dalla cromatografia su colonna venne sciolto in etile acetato ed addizionato di un equivalente molare di acido metansolfonico (sol. 0,5 M in etile acetato). Dopo una notte a 3°C, il prodotto del titolo cristallizzato fu recuperato mediante filtrazione (21%). P.f. 194-195°C.

^{1}H-NMR (DMSO-d_6, δ): 9,35-9,55 (sa, 1H, NH+), 7,12-7,40 (m, 10H, protoni fenilici), 6,75 (dd, 1H, H7 benzodiossanico), 6,50 e 6,58 (2dd, 2H, H6,H8 benzodiossanici), 4,18-4,28 (m, 4H, OCH_2CH_2O), 4,05 (t, 1H, CH), 3,45-3,68 (m, 4H, protoni piperazinici), 2,80-3,30 (m, 6H, protoni piperazinici, $CHCH_2CH_2$), 2,45-2,55 (m, 2H, $CHCH_2CH_2$), 2,30 3H, CH_3S).



ESEMPIO 3

1-[3,3-*bis*-(4-nitrofenil)propil]-4-(2-metossifenil)piperazina dicloridrato . 0,8 H_2O

a) 1-[3,3-*bis*-(4-nitrofenil)propionil]-4-(2-metossifenil)-piperazina (3A)

Il prodotto del titolo fu preparato secondo la metodica descritta nell'esempio 1A a partire da acido 3,3-*bis*-(4-nitrofenil)propionico

(preparato secondo la metodica descritta da Pfeiffer et al., *Annalen* 1953, *581*, 149) invece di acido 3,3-difenilpropionico. Si uso' cloroformio invece di etile acetato per l'estrazione ed il grezzo fu purificato per cristallizzazione da etanolo 80% con ottenimento di un solido fondente a 159-163°C (48%).

^{1}H-NMR ($CDCl_3$, δ): 8,18 (dd, 4H, H3,5 nitrofenilici), 7,42 (dd, 4H, H2,6 nitrofenilici), 6,80-7,14 (m, 4H, aromatici metossifenilici), 4,97 (t, 1H, CH), 3,86 (s, 3H, OCH_3), 3,67-3,78 (m, 2H, CH_2), 3,58-3,67 (m, 2H, $CON(CH\underline{H})_2$ equatoriali), 3,16 (d, 2H, $CON(C\underline{H}H)_2$ assiali), 2,90-3,07 (m, 4H, restanti protoni piperazinici).

b) 1-[3,3-*bis*-(4-nitrofenil)propil]-4-(2-metossifenil)piperazina dicloridrato . 0,8 H_2O

Ad una soluzione di 0,49 g di **3A** in 6 mL di tetraidrofurano anidro agitata in atmosfera di azoto furono aggiunti, a 0-5°C, 1,25 mL di borano-dimetilsolfuro (sol. 2 M in tetraidrofurano). La miscela fu agitata a riflusso per 4 h e raffreddata a 0°C; poi furono aggiunti 1 mL di metanolo e, dopo 0,5 h in agitazione a 20-25°C, 0,5 mL di acido cloridrico (sol. 4 N in isopropanolo). La miscela fu agitata a riflusso per 1 h, diluita con 20 mL di metanolo and evaporata sotto vuoto a secchezza. Il residuo fu ripreso con 10 mL di acqua e la miscela fu resa basica per aggiunta di sodio idrossido 1 N ed estratta con 3x5 mL di cloroformio. Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato anidro ed evaporate sotto vuoto a secchezza. Il residuo fu dissolto in 18 mL di metanolo e la soluzione fu acidificata con acido cloridrico 4 N in isopropanolo in

eccesso. Dopo 3 h a 0°C il cristallizzato fu raccolto per filtrazione a dare 0,31 g (55.7%) del prodotto del titolo fondente a 191-194°C e contenente 0,8 moli di acqua.

^{1}H-NMR ($CDCl_3$, δ): 11,25-11,45 (sa, 1H, NH+), 8,20 (dd, 4H, H3,5 nitrofenilici), 7,70 (dd, 4H, H2,6 nitrofenilici), 6,85-7,07 (m, 4H, aromatici metossifenilici), 5,85-6,18 (sa, 2,6H, H_2O and NH+), 4,54 (t, 1H, CH), 3,77 (s, 3H, OCH_3), 3,55-3,65 (m, 4H, protoni piperazinici), 3,07-3,25 (m, 4H, protoni piperazinici), 2,90-3,07 (m, 2H, $CHCH_2\underline{CH_2}N$), 2,63-2,80 (m, 2H, $CH\underline{CH_2}CH_2N$).

ESEMPIO 4

1-[3,3-*bis*-(4-metossifenil)propil]-4-(2-metossifenil)piperazina dicloridrato

a) 1-[3,3-*bis*-(4-metossifenil)propionil]-4-(2-metossifenil)-piperazina cloridrato (4A)

Il prodotto del titolo fu preparato secondo la metodica descritta nell'esempio 1A a partire da 0,57 g di acido 3,3-*bis*-(4-metossifenil)-propionico (preparato secondo la metodica descritta da Klemm L.H., *J. Org. Chem.* **1958**, *23*, 344) invece di acido 3,3-difenilpropionico. Si usò dietil etere invece di etile acetato per l'estrazione e l'estratto, dopo anidrificazione su sodio solfato anidro, fu acidificato con acido cloridrico (sol. 3 N in dietil etere). Il precipitato fu raccolto per filtrazione e ricristallizzato da acetone a dare 0,65 g del prodotto del titolo (resa 65.5%) fondente a 175-179°C.

^{1}H-NMR (DMSO-d_6, δ): 9,50 (sa, 1H, NH+), 7,15-7,25 (m, 4H, aromatici metossifenilici sistema AA'BB'), 6,88-7,25 (m, 4H, protoni

metossifenilici), 6,76-6,85 (m, 4H, aromatici metossifenilici sistema AA'BB'), 4,38 (t, 1H, CH), 3,82 (s, 3H, OCH_3), 3,55-3,80 (m, 4H, protoni piperazinici), 3,67 (s, 6H, 2 OCH_3), 2,88-3,15 (m, 6H, protoni piperazinici, $C(O)CH_2$).

b) 1-[3,3-*bis*-(4-metossifenil)propil]-4-(2-metossifenil)piperazina dicloridrato

Il prodotto del titolo fu ottenuto seguendo la procedura riportata nell'esempio 3 utilizzando l'intermedio **4A** invece di 3A. Si uso' etile acetato invece di cloroformio per l'estrazione. Il residuo fu dissolto in dietil etere e, dopo trattamento con carbone, la soluzione fu acidificata con acido cloridrico in eccesso (sol. 3 N in dietil etere). Dopo 3 h il precipitato fu raccolto per filtrazione a dare il composto del titolo fondente a 163-171°C.

^{1}H-NMR (DMSO-d_6, δ): 8,80-9,60 (sa, 2H, NH+), 7,18-7,30 (m, 4H, aromatici metossifenilici sistema AA'BB'), 6,80-7,05 (m, 8H, protoni metossifenilici e restanti aromatici metossifenilici sistema AA'BB'), 3,92 (t, 1H, CH), 3,78 (s, 3H, OCH_3), 3,71 (s, 6H, 2 OCH_3), 3,35-3,62 (m, 4H, protoni piperazinici), 3,03-3,25 (m, 4H, protoni piperazinici), 2,85-3,03 (m, 2H, $C\underline{H}_2CH_2CH$), 2,42-2,52 (m, 2H, $CH_2C\underline{H}_2CH$).

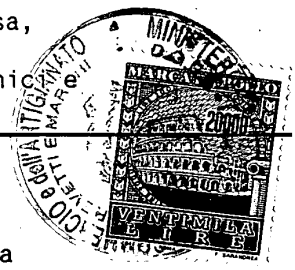
ESEMPIO 5

1-[N,N-*bis*-(2-piridil)-2-amminoetil]-4-(2-metossifenil)piperazina cloridrato

Ad una soluzione di 1,71 g di *bis*-(2-piridil)ammina in 50 mL di toluene agitata a temperatura ambiente furono aggiunti 0,55 g di sodio ammide 95% e, successivamente, 2,54 g di 1-(2-cloroetil)-4-(2-metossi-

fenil)piperazina. La miscela di reazione fu agitata a riflusso per 24 h, raffreddata a temperatura ambiente, diluita cautamente con 10 mL di metanolo e, dopo 15 minuti in agitazione, con 20 mL di acqua e 20 mL di etile acetato. Dopo 10 minuti in agitazione, si separarono le fasi e la fase acquosa fu riestratta con etile acetato. Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (gradiente etere di petrolio - etile acetato - soluzione 2,2 N di ammoniaca in metanolo da 6:4:0,2 a 4:6:0,2). Le frazione raccolte furono evaporate a secchezza 2,51 g di prodotto del titolo come base (64,5%). Questo fu disciolto in 45 mL di etile acetato, al quale fu aggiunto 1 equivalente molare di acido cloridrico (sol. 1 M in etanolo). Dopo una notte a 0°C il prodotto del titolo cristallizzato venne recuperato per filtrazione. P.f. 218-220°C.

^{1}H-NMR (DMSO-d_6, δ): 8,40 (dd, 2H, H6 piridinici), 7,74 (ddd, 2H, H4 piridinici), 7,28 (dd, 2H, H3 piridinici), 6,90-7,15 (m, 6H, H5 piridinici, protoni fenilici), 4,58 (t, 2H, $PyNCH_2$), 4,35-5,15 (sa, 1H, NH+), 3,80 (s, 3H, OCH_3), 2,95-3,35 (m, 10H, protoni piperazinici, $PyNCH_2\underline{CH_2}$).



ESEMPIO 6

1-[3-ciano-3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina

Ad una sospensione di 0,21 g di sodio ammide 95% in 2 mL di 1,2-dimetossietano fu aggiunta per gocciolamento una soluzione di 0,78 g di 2,2-*bis*-(2-piridil)acetonitrile (preparato come descritto in *Heterocycles* 1995, *40*, 757) in 8 mL di 1,2-dimetossietano, mantenendo

la miscela in agitazione in atmosfera di azoto a temperatura ambiente. Dopo 1 h furono gocciolati 1,02 g di 1-(2-cloroetil)-4-(2-metossifenil)piperazina disciolti in 4 mL di 1,2-dimetossietano. La miscela di reazione fu agitata a riflusso per 20 h, raffreddata a temperatura ambiente, versata cautamente in 40 g di ghiaccio, diluita con acqua ed estratta con etile acetato. Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato anidro ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (gradiente etile acetato-metanolo da 10:0 a 9:1). Le frazione raccolte furono evaporate a secchezza a dare 1,13 g di prodotto del titolo (68,4%).

^{1}H-NMR ($CDCl_3$, d): 8,60 (dd, 2H, H6 piridinici), 7,58-7,73 (m, 4H, H3,4 piridinici), 7,22 (ddd, 2H, H5 piridinici), 6,83-7,03 (m, 4H, aromatici metossifenilici), 3,84 (s, 3H, OCH_3), 2,85-3,08 (m, 6H, protoni piperazinici, $CC\underline{H}_2CH_2N$), 2,55-2,70 (m, 6H, protoni piperazinici, $CCH_2C\underline{H}_2N$).

ESEMPIO 7

1-[3-ciano-3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)piperazina dicloridrato

Il prodotto del titolo fu preparato secondo la metodica descritta nell'esempio 6 a partire da 1,86 g di 2-fenil-2-(2-piridil)acetonitrile (preparato come descritto in *Helv. Chim. Acta* 1944, *27*, 1748) invece che da 2,*bis*-(2-piridil)acetonitrile. Il grezzo fu purificato mediante cromatografia flash (etile acetato-etere di petrolio 6:4) a dare, dopo evaporazione sotto vuoto delle frazioni raccolte, 3,39 g (86%) di

prodotto del titolo come base. Questo fu disciolto in 20 mL di etanolo, al quale furono aggiunti 6 mL di acido cloridrico (sol. 5 M in isopropanolo). Dopo una notte a temperatura ambiente vennero recuperati per filtrazione 3,45 g del prodotto del titolo cristallizzato. P.f. 228-230°C.

^{1}H-NMR (DMSO-d_6, δ): 11,50-11,75 (sa, 1H, NH+), 8,65 (dd, 2H, H6 piridinico), 8,25-8,60 (sa, 1H, NH+), 8,40 (ddd, 2H, H4 piridinico), 7,45-7,60 (m, 7H, H3,5 piridinici, protoni fenilici), 6,85-7,10 (m, 4H, aromatici metossifenilici), 3,77 (s, 3H, OCH_3), 3,00-3,75 (m, 12H, protoni piperazinici e CH_2CH_2).

ESEMPIO 8

1-[3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina

Una miscela di 2,44 g del prodotto dell'esempio 6 e 12 mL di acido solforico 70% venne agitata a 125°C per 1,5 h. La miscela di reazione fu poi raffreddata a temperatura ambiente, versata cautamente in 100 g di ghiaccio, diluita con acqua, alcalinizzata con sodio idrossido 35% ed estratta con etile acetato (3x40mL). Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato anidro ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (etile acetato-sol. 2,2 N di ammoniaca in metanolo 9,6:0,4). Le frazione raccolte furono evaporate a secchezza a dare 1,87 g di prodotto del titolo come base (82%).

^{1}H-NMR ($CDCl_3$, δ), 8,55 (dd, 2H, H6 piridinici), 7,58 (ddd, 2H, H4 piridinici), 7,36 (dd, 2H, H3 piridinici), 7,10 (ddd, 2H, H5, piridinici), 6,79-7,03 (m, 4H, aromatici metossifenilici), 4,37 (t, 1H,

CH), 3,84 (s, 3H, OCH_3), 2,95-3,12 (m, 4H, protoni piperazinici), 2,55-2,73 (m, 4H, protoni piperazinici), 2,30-2,55 (m, 4H, CCH_2CH_2N).

ESEMPIO 9

1-[3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)piperazina ed

ESEMPIO 10

1-[3-carbamoil-3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)-piperazina

Una miscela di 1,26 g del prodotto dell'esempio 7 e 6,2 mL di acido solforico 70% venne agitata a 125°C per 40 min. La miscela di reazione fu poi raffreddata a temperatura ambiente, versata cautamente in 60 g di ghiaccio, diluita con acqua, basificata con sodio idrossido 35% ed estratta con etile acetato (2x60mL). Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (gradiente etile acetato-etere di petrolio-sol. 2,7 N di ammoniaca in metanolo da 5:5:0,5 a 8:2:0,5). Dall'evaporazione sotto vuoto delle frazioni meno polari furono ottenuti 0,25 g del prodotto dell'Esempio 9.

~~^{1}H-NMR ($CDCl_3$, δ): 8,59 (dd, 1H, H6 piridinico), 7,54 (ddd, 1H, H4~~ piridinico), 7,08-7.41 (m, 7H, H3,5 piridinici, aromatici fenilici), 6,82-7,07 (m, 4H, aromatici metossifenilici), 4,18 (t, 1H, $C\underline{H}CH_2$), 3,85 (s, 3H, OCH_3), 3,00-3,15 (m, 4H, protoni piperazinici), 2,25-2,73 (m, 8H, protoni piperazinici e CH_2CH_2).

Dall'evaporazione delle frazioni piu' polari furono ottenuti 0,78 g del prodotto dell'Esempio 10 come olio. Quest'ultimo fu

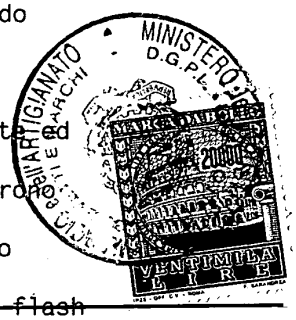
cristallizzato da acetonitrile a dare, dopo filtrazione, 0,35 g di un solido fondente a 156-164°C.

^{1}H-NMR ($CDCl_3$, δ): 9,20-9,40 (sa, 1H, $CONH_2$), 8,55 (dd, 1H, H6 piridinico), 7,60 (ddd, 1H, H4 piridinico), 7,10-7,35 (m, 7H, H3,5 piridinici, aromatici fenilici), 6,80-7,05 (m, 4H, aromatici metossifenilici), 5,60-5,75 (sa, 1H, $CONH_2$), 3,83 (s, 3H, OCH_3), 2,15-3,15 (m, 12H, protoni piperazinici e CH_2CH_2).

ESEMPIO 11

1-[N-(2-nitrofenil)-N-(2-piridil)-2-amminoetil]-4-(2-metossifenil)-piperazina

Una miscela di 0,43 g di 1-[N-(2-nitrofenil)-2-amminoetil]-4-(2-metossifenil)piperazina (preparata come descritto in US 3,472,854), 0,19 g di 2-bromopiridina, 0,17 g di potassio carbonato anidro e 0,01 g di rame in polvere fu scaldata a 100°C per 3 h. Dopo questo periodo furono aggiunti altri 0,138 g di 2-bromopiridina e la miscela fu scaldata a 160°C per altre 24 h, raffreddata a temperatura ambiente ed estratta con etile acetato (2x20mL). Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (etile acetato-etere di petrolio 7:3). Le frazione raccolte furono evaporate a secchezza a dare 0.25 g (52%) di prodotto del titolo.



^{1}H-NMR ($CDCl_3$, δ): 8,12 (dd, 1H, H6 piridinico), 7,98 (dd, 1H, H3 nitrofenilico), 7,52-7,70 (m, 2H, aromatici), 7,30-7,50 (m, 2H, aromatici), 6,79-7,03 (m, 4H, aromatici metossifenilici), 6,65 (dd, 1H, H5 piridinico), 6,33 (dd, 1H, H3 piridinico), 4,08 (t, 2H, CH_2NPy),

3,84 (s, 3H, OCH_3), 2,90-3,05 (m, 4H, protoni piperazinici), 2,80 (t, 2H, CH_2CH_2NPy), 2,60-2,75 (m, 4H, protoni piperazinici).

ESEMPIO 12

1-[3-ciano-3-(2-nitrofenil)-3-fenilpropil]-4-(2-metossifenil)piperazina

a) 1-(3-ciano-3-fenilpropil)-4-(2-metossifenil)piperazina (12A)

Il prodotto del titolo fu sintetizzato secondo la metodica descritta nell'esempio 6 sostituendo il 2,2-*bis*-(2-piridil)acetonitrile con 0,59 g di fenilacetonitrile ed il 1,2-dimetossietano con toluene. La miscela di reazione fu agitata a 80°C per 3,5 h. Il grezzo fu purificato mediante cromatografia flash (etile acetato-etere di petrolio 6:4). Le frazione raccolte furono evaporate a secchezza a dare 0,96 g di prodotto del titolo (57,3%).

^{1}H-NMR ($CDCl_3$, δ): 7,35-7,45 (m, 5H, aromatici fenilici), 6,79-7,03 (m, 4H, aromatici metossifenilici), 4,08 (t, 1H, CH), 3,86 (s, 3H, OCH_3), 3,05-3,20 (m, 4H, protoni piperazinici), 2,38-2,70 (m, 6H, protoni piperazinici, 2H del CH_2CH_2), 1,95-2,35 (m, 2H, 2H del CH_2CH_2).

b) 1-[3-ciano-3-(2-nitrofenil)-3-fenilpropil]-4-(2-metossifenil)-piperazina

Una miscela di 0,24 g del prodotto 12A, 0,11 g di 2-cloro-nitrobenzene, 0,5 mL di sodio idrossido 50%, 0,02 g di trietil benzilammonio cloruro e 0,5 mL di toluene fu agitata a 60°C per 6 h. Successivamente la miscela fu raffreddata a temperatura ambiente, diluita con 20 mL di acqua ed estratta con 2x20 mL di etile acetato. Le fasi organiche riunite furono lavate con acqua, anidrificate su

sodio solfato ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (gradiente etere di petrolio - etile acetato 5:5). Le frazione raccolte furono evaporate a secchezza a dare 0,12 g (36%) di prodotto del titolo. Questo fu disciolto in cloruro di metilene, evaporato sotto vuoto a secchezza ed essiccato sotto vuoto (1 mmHg) a dare un solido fondente a 61-64°C.

^{1}H-NMR ($CDCl_3$, δ): 8,05 (dd, 1H, H3 nitrofenilico), 7,50-7,73 (m, 3H, H4,5,6 nitrofenilici), 7,20-7,35 (m, 5H, aromatici fenilici), 6,79-7,03 (m, 4H, aromatici metossifenilici), 3,84 (s, 3H, OCH_3), 2,95-3,15 (m, 5H, protoni piperazinici, C<u>H</u>HCH_2N), 2,35-2,75 (m, 7H, protoni piperazinici, CH<u>H</u>C<u>H</u>$_2$N).

ESEMPIO 13

1-[3-carbamoil-3-(2-nitrofenil)-3-fenilpropil]-4-(2-metossifenil)-piperazina

Il prodotto del titolo fu ottenuto seguendo la metodica descritta nell'esempio 8, a partire da 0,21 g del prodotto dell'esempio 12 invece che dal prodotto dell'esempio 6 e scaldando a 125°C per 105 minuti. Dopo l'usuale lavorazione, il grezzo fu purificato mediante cromatografia flash (etile acetato-metanolo 95:5). Le frazione raccolte furono evaporate a secchezza a dare 0,1 g di prodotto del titolo come olio (46%).

^{1}H-NMR ($CDCl_3$, δ): 7,75-7,82 (m, 1H, H3 nitrofenilico), 7,55-7,80 (m, 1H, $CONH_2$), 7,25-7,50 (m, 7H, aromatici fenilici, H 4,5 nitrofenilici), 7,05-7,15 (m, 1H, H6 nitrofenilico), 6,79-7,03 (m, 4H, aromatici metossifenilici), 5,30-5,55 (m, 1H, $CONH_2$), 3,84 (s, 3H,

OCH_3), 3,00-3,15 (m, 4H, protoni piperazinici), 2,25-2,95 (m, 8H, protoni piperazinici, CH_2CH_2).

ESEMPIO 14

1-[3-idrossi-3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina

Ad una soluzione di 0,17 mL di 2-bromopiridina in 6 mL di tetraidrofurano agitata a -50°C in atmosfera di azoto furono addizionati per gocciolamento in 5 minuti 0,72 mL di butil litio (soluzione 2,5 M in esano). Dopo 6 minuti a -55°C fu gocciolata in 10 minuti una soluzione di 0,5 g di 3-[4-(2-metossifenil)-1-piperazinil]-propionato di etile (preparato come descritto in DE 2,555,290) in 3 mL di tetraidrofurano anidro. Dopo 1,5 h a -50°C, la reazione fu spenta per aggiunta di una soluzione satura di ammonio cloruro. La miscela fu estratta con 2x50 mL di etile acetato. Le fasi organiche riunite furono lavate con acqua, anidrificate su sodio solfato ed evaporate sotto vuoto a secchezza. Il grezzo fu purificato mediante cromatografia flash (etile acetato-sol. 3,8 N di ammoniaca in metanolo 99:1). Le frazione raccolte furono evaporate a secchezza a dare 0,11 g di prodotto del titolo (15%).

^{1}H-NMR ($CDCl_3$, δ): 8,56 (dd, 2H, H6 piridinici), 7,79 (dd, 2H, H3 piridinici), 7,64 (ddd, 2H, H4 piridinici), 7,10 (ddd, 2H, H5 piridinici), 6,85-7,03 (m, 4H, aromatici metossifenilici), 3,84 (s, 3H, OCH_3), 2,95-3,12 (m, 4H, protoni piperazinici), 2,76 (t, 2H, $C(OH)C\underline{H}_2CH_2$), 2,55-2,75 (m, 4H, protoni piperazinici), 2,50 (t, 2H, $C(OH)CH_2C\underline{H}_2$).

Attivita' dei composti dell'invenzione

L'attivita' dei composti dell'invenzione come inibitori della frequenza di minzione e per migliorare la capacita' della vescica li rende efficaci per la terapia delle disfunzioni neuromuscolari delle basse vie urinarie nei mammiferi, tra le quali, senza limitazioni, disuria, incontinenza ed enuresi.

Le caratteristiche dei composti dell'invenzione conferiscono loro una potenza nettamente maggiore rispetto agli standard di riferimento rappresentati dai farmaci riportati in precedenza: flavossato e imipramina, e l'ossibutinina che evidenzia un profilo d'azione diverso.

Tali dati sono stati raccolti sperimentando i suddetti composti in un modello di ratto in cui è stata indotta la contrazione ritmica della vescica riempiendola con soluzione fisiologica ed è stato valutato l'effetto dei composti in esame e degli standard di riferimento sulla frequenza e l'ampiezza delle contrazioni, con particolare riguardo alla potenza nell'indurre la scomparsa delle contrazioni ritmiche.

Prima della presente invenzione, il trattamento delle disfunzioni neuromuscolari delle basse vie urinarie comportava la somministrazione di composti che agiscono direttamente sulla muscolatura della vescica, come il flavossato, farmaco spasmolitico attivo anche sul centro pontino della minzione, composti anticolinergici come l'ossibutinina, e farmaci ad azione mista come l'imipramina (Andersson K. E., *Drugs of Today* 24(5), 337-348 (1988)).

Tuttavia, le terapie che comportano l'inibizione diretta della muscolatura pelvica (compreso il detrusore) possono avere effetti

collaterali indesiderabili quali svuotamento incompleto o paralisi da accomodazione, tachicardia e secchezza della bocca (Andersson, *Drugs* 35:477, 1988) e farmaci come l'imipramina possono avere effetti tossici rilevanti, in particolare sull'apparato cardiovascolare (ipotensione ortostatica, aritmia ventricolare), alle dosi terapeutiche. Sarebbe quindi preferibile aumentare il numero di farmaci a disposizione del medico per il trattamento della funzionalita' neuromuscolare delle basse vie urinarie. Anche l'effetto dei farmaci disponibili attualmente (flavossato, ossibutinina e imipramina) sul suddetto modello di ratto è riportato nella Tabella 1.

I composti dell'invenzione evidenziano una maggiore potenza nella durata d'azione (per esempio durata della quiescenza della vescica senza contrazioni rappresentata in Tabella 1 dalla $ED_{10\ min}$) rispetto al flavossato, all'ossibutinina e all'imipramina. Inoltre, diversamente dall'ossibutinina, i composti dell'invenzione non incidono sull'ampiezza delle contrazioni, a indicazione che non c'è compromissione della contrattilita' della vescica.

Infine la presenza di un'affinita' elevata per il recettore $5\text{-}HT_{1A}$ (Tabella 2) indica un ruolo importante di questo recettore nell'azione dei composti dell'invenzione. I test farmacologici (e le Tabelle) suddetti sono riportati nella parte Dati Farmacologici descritta di seguito.

Applicazioni terapeutiche

I pazienti che necessitano di trattamento con questi composti e composizioni sono affetti da disfunzioni neuromuscolari delle basse vie

urinarie trattate da E.J.McGuire in "Campbell's UROLOGY" 5° Ed., 616-638, 1986, W.B. Saunders Company, e sono inoltre quelli affetti dalle disfunzioni associate alla compromissione della funzionalita' del recettore 5-HT_{1A}.

La presente invenzione comprende le formulazioni farmaceutiche che contengono i composti sopra elencati, nonche' i metodi che impiegano tali formulazioni per il trattamento delle disfunzioni neuromuscolari delle basse vie urinarie quali disuria, incontinenza ed enuresi. La disuria comprende la frequenza urinaria, la nicturia e l'impellenza. Le sindromi d'incontinenza comprendono incontinenza da sforzo, incontinenza da impellenza e incontinenza da travaso. L'enuresi riguarda il passaggio involontario di urina la notte o durante il sonno.

Senza voler essere legati dalla teoria, si ritiene che la somministrazione di antagonisti del recettore 5-HT_{1A} prevenga l'attivita' indesiderata dell'arco riflesso sacrale e/o dei meccanismi corticali che regolano la minzione. Si prevede quindi che un'ampia gamma di disfunzioni neuromuscolari delle basse vie urinarie possano essere trattate con i composti della presente invenzione.

La "quantita' efficace" di composto per trattare i disturbi urinari è una quantita' che produce un miglioramento apprezzabile di almeno un sintomo o parametro del disturbo.

I disturbi delle vie urinarie e i relativi sintomi sono urgenza, frequenza, incontinenza, perdita di urina, enuresi, disuria, difficolta' di minzione e difficolta' di svuotamento della vescica.

Un ulteriore parametro è il volume di urina. La quantita' efficace per trattare il disturbo puo' essere trovata con esperimenti noti nell'arte, come stabilire una matrice di dosi e frequenze e confrontare un gruppo di unita' sperimentali o soggetti in corrispondenza di ciascun punto della matrice. La quantita' esatta da somministrare al paziente puo' variare a seconda dello stato e della gravita' del disturbo e della condizione fisica del paziente, il miglioramento apprezzabile dei sintomi e dei parametri puo' essere stabilito da un medico esperto nell'arte o segnalato dal paziente al medico. E' sottinteso che l'attenuazione clinicamente o statisticamente rilevante di sintomi e parametri rientra negli scopi dell'invenzione. Attenuazione clinicamente rilevante significa percettibile al paziente e/o al medico.

I composti della presente invenzione possono essere formulati in forme farmaceutiche liquide con un veicolo fisiologicamente accettabile come, per esempio, soluzione fisiologica tamponata con fosfato o acqua deionizzata. La formulazione farmaceutica puo' contenere anche eccipienti, compresi conservanti e stabilizzanti, che sono ben noti nell'arte. I composti possono essere formulati in unita' farmaceutiche solide orali o meno quali, per esempio, compresse, capsule, polveri e supposte, e possono inoltre contenere eccipienti, compresi senza limiti lubrificanti, plasticizzanti, coloranti, promotori dell'assorbimento, battericidi e simili.

I modi di somministrazione sono la via orale ed enterale, endovenosa, intramuscolare, sottocutanea, transdermica, transmucosica

(compreso boccale e rettale) e per inalazione. Preferibilmente, si usa la via orale o transdermica (ossia, rispettivamente con formulazioni orali solide o liquide o con cerotti cutanei).

La quantita' di agente da somministrare puo' variare da circa 0,01 a circa 25 mg/kg/die, preferibilmente da circa 0,1 a circa 10 mg/kg/die e molto preferibilmente circa 0,2-5 mg/kg/die. E' inteso che le formulazioni farmaceutiche della presente invenzione non necessitano di per se' di contenere la quantita' intera di agente efficace nel trattamento del disturbo, poiche' tale quantita' efficace puo' essere raggiunta con la somministrazione di una pluralita' di dosi delle formulazioni farmaceutiche stesse.

Secondo un aspetto preferito della presente invenzione, i composti sono formulati in capsule o compresse, ciascuna preferibilmente contenente 50-200 mg di composti, e sono molto preferibilmente somministrati al paziente alla dose giornaliera totale di 50-400 mg, preferibilmente 150-250 mg, e molto preferibilmente 200 mg, per la cura dell'incontinenza urinaria e delle disfunzioni associate alla compromissione dei recettori $5\text{-}HT_{1A}$.

I metodi, le tabelle e gli esempi forniti di seguito hanno lo scopo di illustrare piu' esaurientemente le versioni preferite dell'invenzione e di dimostrare i suoi vantaggi e la sua applicabilita', ma senza limitarne la portata.

Dati farmacologici

Effetti sulle contrazioni ritmiche di svuotamento vescicale indotte dal volume nel ratto anestetizzato

A. Metodi:

Sono stati usati ratti Sprague Dawley femmine del peso di 225-275 g (Crl: CDo BR, Charles River, Italia). Gli animali sono stati alloggiati con libero accesso a cibo e acqua e mantenuti in un ciclo forzato di 12 ore di alternanza luce/buio a 22-24°C per almeno una settimana, tranne durante l'esperimento. L'attivita' sulle contrazioni ritmiche di svuotamento vescicale è stata valutata secondo il metodo di Dray (J. Pharmacol. Methods, 13:157, 1985), con alcune modifiche come in Guarneri (Pharmacol. Res., 27:173, 1993). In breve, i ratti sono stati anestetizzati con iniezione sottocutanea di 1,25 g/kg (5 ml/kg) di uretano, dopo di che è stata cateterizzata la vescica attraverso l'uretra usando un tubo di polietilene PE 50 riempito di soluzione fisiologica. Il catetere è stato fissato in posizione con una legatura attorno all'orifizio uretrale esterno e collegato a un comune trasduttore di pressione (Statham P23 ID/P23 XL). La pressione endovescicale è stata visualizzata in continuo su un registratore a nastro (Battaglia Rangoni KV 135 con amplificatore DC1/TI). E' stata quindi riempita la vescica attraverso il catetere di registrazione con volumi incrementali di soluzione fisiologica tiepida (37°C) fino a comparsa delle contrazioni riflesse di svuotamento (in genere 0,8-1,5 ml). Per l'iniezione endovenosa (i.v.) dei composti allo studio, è stato inserito nella vena giugulare un tubo di polietilene PE 50 riempito di soluzione fisiologica.

Dal cistometrogramma sono stati ricavati il numero di contrazioni registrate 15 minuti prima (valore basale) e dopo il trattamento e

l'ampiezza media delle contrazioni stesse (altezza media del picco in mmHg).

Poiche' la maggior parte dei composti produce un effetto che insorge con relativa rapidita' e porta alla completa cessazione delle contrazioni vescicali, la bioattivita' è stata valutata facilmente misurando la durata della quiescenza della vescica (ossia, il tempo in cui non vi sono contrazioni). Inoltre, è stato registrato il numero di animali esaminati che evidenzia una riduzione del numero di contrazioni > 30% rispetto a quelle osservate nel periodo basale.

Per confrontare la potenza dei composti sperimentati nell'inibire le contrazioni di svuotamento vescicale, sono state calcolate le dosi equiefficaci che producono 10 minuti di tempo di scomparsa (ED_{10min}) mediante analisi della regressione lineare col metodo dei minimi quadrati, nonche' le dosi estrapolate che inducono una riduzione del numero di contrazioni > 30% nel 50% dei ratti trattati (ED_{50}, frequenza) con il metodo di Bliss (Bliss C.I., Quart. J. Pharm. Pharmacol. 11, 192-216, 1938). Dopo la cessazione dell'effetto dell'iniezione di farmaco, e' stata confrontata l'altezza dei picchi con quella precedentemente registrata dopo la somministrazione endovenosa del solo veicolo. E' stata valutata la potenza dei composti esaminati (valore ED_{50}: dosi estrapolate che inducono il 30% di riduzione dell'ampiezza delle contrazioni nel 50% dei ratti trattati) su base quantitativa col metodo di Bliss (Bliss C.I., Quart. J. Pharm. Pharmacol. 11, 192-216, 1938).

B. Risultati

La rapida distensione della vescica nel ratto anestetizzato con uretano produce una serie di contrazioni ritmiche di svuotamento le cui caratteristiche sono pubblicate (Maggi et al., Brain Res., 380:83, 1986; Maggi et al., J. Pharmacol. Exp. Ther., 230:500, 1984). La frequenza delle contrazioni e' associata al braccio sensitivo afferente del riflesso della minzione e all'integrita' del centro della minzione, mentre la loro ampiezza e' una proprieta' del braccio efferente del riflesso. In questo modello, i composti che agiscono principalmente sul sistema nervoso centrale (come la morfina) provocano un blocco delle contrazioni di svuotamento, mentre i farmaci che agiscono a livello del detrusore, come l'ossibutinina, riducono l'ampiezza delle contrazioni. La tabella 1 riporta i risultati ottenuti con i composti in esame.

TABELLA 1

Effetti sulle contrazioni ritmiche di svuotamento vescicale dopo somministrazione endovenosa

Composto	ED_{10min} µg/kg	ED_{50} (frequenza) µg/kg	ED_{50} (ampiezza) µg/kg
Esempio 5	523	77	n.a.
Esempio 6	225	93	n.a.
Esempio 7	78	18	n.a.
Esempio 8	74	2.5	n.a.
Esempio 9	77	25	n.a.
Esempio 10	228	180	n.a.
Flavossato	>10000	2648	n.a.
Ossibutinina	7770	10000	240
Imipramina	>6000	1676	2930

n.a. = non attivo; nessuna riduzione importante dell'altezza dei picchi.

I dati rappresentano i valori di ED_{10min} (dose estrapolata che induce 10 minuti di scomparsa delle contrazioni); i valori di ED_{50} (frequenza) (dose estrapolata che induce una riduzione del numero di contrazioni > 30% nel 50% dei ratti trattati) e i valori di ED_{50} (ampiezza) (dose estrapolata che induce una riduzione > 30% dell'ampiezza delle contrazioni nel 50% dei ratti trattati).

Tutti i composti della presente invenzione sono nettamente piu' potenti del flavossato, dell'ossibutinina e dell'imipramina nell'inibire le contrazioni di svuotamento se si tiene conto sia dell'ED_{10min} sia dell'ED_{50}. Inoltre, diversamente dall'ossibutinina e dall'imipramina e analogamente al flavossato, non incidono sull'ampiezza delle contrazioni, a indicazione che non vi e' compromissione della contrattilita' vescicale.

Legame radiorecettoriale al recettore $5\text{-}HT_{1A}$ e altri siti di legame di diversi neurotrasmettitori

A. Metodi

- ***Recettori $5HT_{1A}$ ricombinanti umani***

La codifica genomica del clone G-21 del recettore serotoninergico $5\text{-}HT_{1A}$ umano e' stabilmente trasferita in una linea cellulare umana (HeLa). Sono state coltivate cellule HeLa in monostrati in terreno Eagle modificato di Dulbecco (DMEM) integrato con siero di feto di vitello al 10% e gentamicina (100 mg/ml), CO_2 al 5% a 37°C. Le cellule sono state staccate dalla fiasca di coltivazione al 95% di confluenza

con un raccoglitore cellulare e sottoposte a lisi in tampone ghiacciato Tris 5mM ed EDTA 5mM (pH 7,4). Gli omogenati sono stati centrifugati a 40000 x g per 20 minuti e le membrane sono state risospese in un piccolo volume di tampone ghiacciato Tris 5mM ed EDTA 5mM (pH 7,4) e immediatamente congelate e conservate a -70°C fino al momento dell'uso.

Il giorno dell'esperimento le membrane cellulari sono state risospese in tampone legante: Tris-HCl 50 mM (pH 7,4), $MgCl_2$ 2,5mM, pargilina 10µM (Fargin et al., Nature 335, 358-360, 1988). Le membrane sono state incubate in un volume finale di 1 ml per 30 minuti a 30°C con [^{3}H]8-OH-DPAT 0,2-1 nM in assenza o presenza di sostanze in esame; il legame non specifico e' stato stabilito in presenza di 5-HT 10 µM.

L'incubazione e' stata arrestata con l'aggiunta di tampone Tris-HCl ghiacciato e filtrazione rapida attraverso filtri Whatman GF/B o Schleicher & Schuell GF52 pretrattati con polietilenimmina 0,2%.

- *Recettori 5-HT_{2A} serotoninergici nativi (tessuti animali)*

Gli studi di legame ai recettori serotoninergici 5-HT_{2A} nativi (Craig A. e Kenneth J., Life Sci. 38, 117-127, 1986) sono stati eseguiti con membrana di corteccia cerebrale di ratto. Dei ratti maschi Sprague-Dawley (200-300 g, SD Harlan/Nossan, Italia) sono stati uccisi per lussazione cervicale e ne sono state asportate, immediatamente congelate e conservate a -70°C fino al momento dell'uso le cortecce cerebrali. I tessuti sono stati omogenati (2 x 20 sec.) in 50 volumi di tampone freddo Tris-HCl 50 mM pH 7,4 con un omogenizzatore Politron (velocita' 7). Gli omogenati sono stati centrifugati a 49000 x g per 10 minuti, risospesi in 50 volumi dello stesso tampone, incubati a 37°C

per 15 minuti e centrifugati e risospesi altre due volte. Le membrane finali sono state sospese in 100 volumi di tampone Tris-HCl 50 mM pH 7,7. Le membrane sono state incubate in un volume finale di 1 ml per 20 minuti a 37°C con [^{3}H]Ketanserina 0,7-1,3 nM in assenza o presenza di sostanze spiazzanti. Il legame non specifico e' stato stabilito in presenza di ketanserina 2 µM. L'incubazione e' stata fermata con l'aggiunta di tampone Tris-HCl 50 mM ghiacciato e filtrazione rapida attraverso filtri Whatman GF/B o Schleicher & Schuell GF52 pretrattati con polietilenimmina 0,2%. I filtri sono stati poi lavati con tampone ghiacciato e la radioattivita' trattenuta dai filtri e' stata valutata mediante spettrometria per scintillazione liquida.

- *Recettori α_1 adrenergici nativi (tessuti animali)*

Gli studi di legame sui recettori α_1 adrenergici nativi sono stati eseguiti con membrana di corteccia cerebrale di ratto. Dei ratti maschi Sprague Dawley (200-300 g, Charles River, Italia) sono stati uccisi per lussazione cervicale e ne sono state dissecate, immediatamente congelate e conservate a -70°C fino al momento dell'uso le cortecce cerebrali. I tessuti sono stati omogenati (2 x 20 sec.) in 50 volumi di tampone freddo Tris-HCl 50 mM pH 7,4 con un omogenizzatore Politron (velocita' 7). Gli omogenati sono stati centrifugati a 48000 x g per 10 minuti, risospesi in 50 volumi dello stesso tampone, incubati a 37°C per 15 minuti e centrifugati e risospesi altre due volte. Le membrane finali sono state sospese in 100 volumi di tampone Tris-HCl 50 mM pH 7,4 contenenti pargilina 10 µM e acido ascorbico 0,1%. Le membrane sono state incubate in un volume finale di 1 ml per 30 minuti

a 25°C con [^{3}H]prazosina 0,1-0,5 nM in assenza o presenza di sostanze spiazzanti. Il legame non specifico e' stato stabilito in presenza di fentolamina 10 µM. L'incubazione e' stata fermata con l'aggiunta di tampone Tris-HCl 50 mM ghiacciato e filtrazione rapida attraverso filtri Whatman GF/B o Schleicher & Schuell GF52 pretrattati con polietilenimmina 0,2%. I filtri sono stati poi lavati con tampone ghiacciato e la radioattivita' trattenuta dai filtri e' stata valutata mediante spettrometria per scintillazione liquida.

Analisi dei dati

Per calcolare il valore di IC_{50} e' stata valutata l'inibizione del legame specifico dei radioleganti da parte delle sostanze studiate per mezzo del programma di interpolazione non lineare Allfit (De Lean et al., Am. J. Physiol. 235, E97-E102, 1978). Il valore IC_{50} e' stato convertito in costante di affinita' (Ki) per mezzo dell'equazione di Cheng & Prusoff (Cheng Y.C., Prusoff W.H., Biochem. Pharmacol. 22, 3099-3108, 1973).

B. Risultati

I risultati sono riportati nella tabella 2 e indicano che i composti della presente invenzione posseggono un'elevata affinita' per il recettore 5-HT_{1A} e selettivita' per questo recettore rispetto ai recettori serotoninergici 5-HT_{2A} e α_1 adrenergici.

TABELLA 2

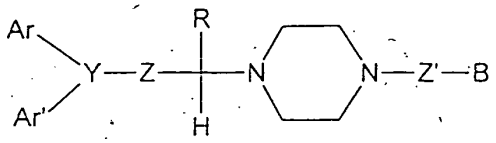
Affinita' di legame per il recettore 5-HT_{1A} e altri siti recettoriali

I dati sono espressi come Ki (nM)

Composto	5-HT_{1A}	5-HT_{2A}	α_1
Esempio 1	3.9	320	145
Esempio 2	0.6	159	208
Esempio 7	7.7	140	396
Esempio 8	3.97	320	191
Esempio 9	0.62	1023	268
Esempio 10	19.3	683	1322
Esempio 12	1.45	-	226
Esempio 14	0.34	-	114

RIVENDICAZIONI

1. Composti di formula generale I



(I)

dove

sia Ar sia Ar' separatamente rappresentano un gruppo arile sostituito o non sostituito o un gruppo eteroarile sostituito o non sostituito,

Y rappresenta un atomo di azoto o un gruppo CH, C-OH, C-CN o $C\text{-}CONH_2$,

R rappresenta un atomo di idrogeno o un gruppo alchile inferiore,

B rappresenta un gruppo arile sostituito o non sostituito o un gruppo eteroarile sostituito o non sostituito,

Z rappresenta un gruppo metilene o etilene, e

Z' rappresenta un legame di valenza o un gruppo metilene o etilene,

e gli enantiomeri, i diastereoisomeri, gli N-ossidi, i polimorfi, i solvatati e i sali farmaceuticamente accettabili di tali composti.

2. Qualsiasi dei seguenti composti:

1-(3,3-difenilpropil)-4-(2-metossifenil)piperazina,

1-(3,3-difenilpropil)-4-[5-(2,3-diidro-1,4-benzodiossinil)]-piperazina,

1-[3,3-*bis*-(4-nitrofenil)propil]-4-(2-metossifenil)piperazina,

1-[3,3-*bis*-(4-metossifenil)propil]-4-(2-metossifenil)piperazina,

1-[N,N-*bis*-(2-piridil)-2-amminoetil]-4-(2-metossifenil)piperazina

1-[3-ciano-3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina,

1-[3-ciano-3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)piperazina,

1-[3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina,

1-[3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)piperazina,

1-[3-carbamoil-3-fenil-3-(2-piridil)propil]-4-(2-metossifenil)-piperazina,

1-[N-(2-nitrofenil)-N-(2-piridil)-2-amminoetil]-4-(2-metossifenil)-piperazina,

1-[3-ciano-3-(2-nitrofenil)-3-fenilpropil]-4-(2-metossifenil)-piperazina,

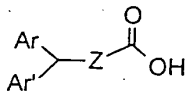
1-[3-carbamoil-3-(2-nitrofenil)-3-fenilpropil]-4-(2-metossifenil)-piperazina, e

1-[3-idrossi-3,3-*bis*-(2-piridil)propil]-4-(2-metossifenil)piperazina.

3. Composizioni farmaceutiche contenenti composti di formula I come definiti nella rivendicazione 1, o enantiomeri, diastereoisomeri, N-ossidi, polimorfi, solvatati o sali farmaceuticamente accettabili di tali composti, in miscela con diluenti o veicoli farmaceuticamente accettabili.

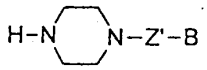
4. Uso delle composizioni secondo la rivendicazione 3, per il trattamento di pazienti affetti da disfunzioni neuromuscolari delle basse vie urinarie, uso che prevede la somministrazione al paziente di una quantita' terapeuticamente efficace di composti di formula generale I come definiti nella rivendicazione 1 o di enantiomeri, diastereoisomeri, N-ossidi, polimorfi, solvatati o sali farmaceuticamente accettabili di tali composti.

5. Procedimento per la preparazione di composti di formula I dove Ar, Ar', B, Z e Z' sono come definiti nella rivendicazione 1, Y rappresenta un gruppo CH e R rappresenta un atomo di idrogeno, metodo che prevede la reazione di un composto II

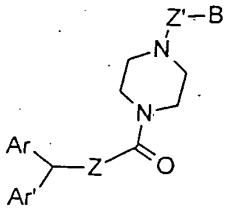


II

dove Ar, Ar' e Z sono come definiti nella rivendicazione 1, con un derivato piperazinico di formula

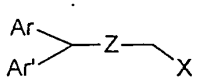


dove Z' e B sono come definiti nella rivendicazione 1 e riduzione del composto ottenuto III



III

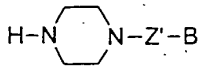
6. Procedimento per la preparazione di composti di formula I dove Ar, Ar', B, Z e Z' sono come definiti nella rivendicazione 1, Y rappresenta un gruppo CH e R rappresenta un atomo di idrogeno, metodo che prevede la reazione di un composto di formula V



V

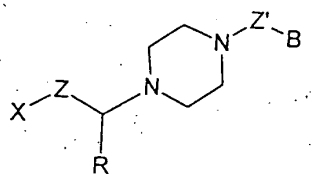
dove Ar, Ar' e Z sono come definiti nella rivendicazione 1 e X

rappresenta un gruppo uscente, come un atomo di alogeno o un gruppo alchilsolfonilossi o arilsolfonilossi, con un derivato piperazinico di formula



dove Z' e B sono come definiti nella rivendicazione 1.

7. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un gruppo CH, metodo che prevede l'alchilazione del carbanione di un composto $ArCH_2Ar'$ dove Ar e Ar' sono come definiti nella rivendicazione 1 con un composto di formula VI



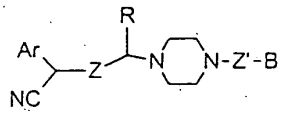
VI

dove R, B, Z e Z' sono come definiti nella rivendicazione 1 ed X e' come definito nella rivendicazione 6.

8. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un gruppo C-CN, metodo che prevede l'alchilazione del carbanione di un composto ArCH(CN)Ar' dove Ar e Ar' sono come definiti nella rivendicazione 1 con un composto di formula VI come definito nella rivendicazione 7.

9. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y

rappresenta un gruppo C-CN, procedimento che prevede la reazione di un composto $ArCH_2CN$ con un composto di formula VI come definito nella rivendicazione 7, e l'arilazione del composto ottenuto IX



IX

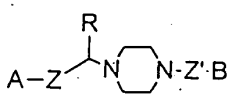
con un composto Ar'- Hal dove Ar' e' come definito nella rivendicazione 1 e Hal rappresenta un atomo di alogeno.

10. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un gruppo $C-CONH_2$, metodo che prevede l'idrolisi di un corrispondente composto I in cui Y rappresenta un gruppo C-CN con un acido acquoso o acido di Lewis a temperatura massima di 80°C.

11. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un gruppo CH, metodo che prevede l'idrolisi di un corrispondente composto I in cui Y rappresenta un gruppo C-CN o $C-CONH_2$ con acido solforico almeno al 70% a temperatura di riflusso.

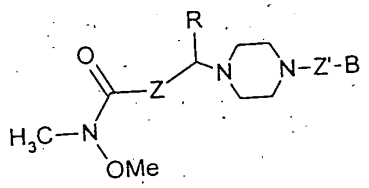
12. Procedimento per la preparazione di composti di formula I dove Ar, R, B, Z e Z' sono come definiti nella rivendicazione 1, Ar' e' lo stesso di Ar e Y rappresenta un gruppo C-OH, metodo che prevede la reazione di un derivato metallico arilico Ar-Met dove Ar e' come definito nella rivendicazione 1 e Met rappresenta un metallo come litio o magnesio con un composto X dove R, B, Z e Z' sono come definiti nella rivendicazione 1 e A rappresenta un gruppo carbossilato, ciano o

carbamoile.



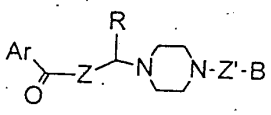
X

13. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un gruppo C-OH, procedimento che prevede la reazione di un derivato metallico arilico Ar-Met con un composto X'



X'

dove R, B, Z e Z' sono come definiti nella rivendicazione 1, e la reazione del composto ottenuto XI



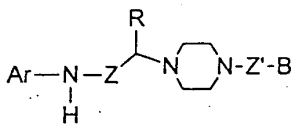
IX

con un derivato metallico arilico Ar'-Met dove Ar' e' come definito nella rivendicazione 1 e Met e' come definito in questa rivendicazione.

14. Procedimento per la preparazione di composti di formula I dove Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un atomo di azoto, metodo che prevede la reazione dell'aza-anione di ArHNAr' dove Ar e Ar' sono come definiti nella rivendicazione 1 con un composto VI, definito nella rivendicazione 7.

15. Procedimento per la preparazione di composti di formula I dove

Ar, Ar', R, B, Z e Z' sono come definiti nella rivendicazione 1 e Y rappresenta un atomo di azoto, metodo che prevede la reazione di un composto Ar'-X dove Ar' e' come definito nella rivendicazione 1 con un composto XIII



XIII

dove Ar, R, B, Z e Z' sono come definiti nella rivendicazione 1.

16. Uso delle composizioni secondo la rivendicazione 3 per il trattamento di pazienti affetti da disturbi del sistema nervoso centrale, quali l'ansia e la depressione, l'ipertensione, i disturbi del ciclo sonno/veglia, il comportamento alimentare e/o la funzionalita' sessuale e i disturbi cognitivi, uso che prevede la somministrazione al paziente di una quantita' terapeuticamente efficace di composti di formula I come definiti nella rivendicazione 1 o di enantiomeri, diastereoisomeri, N-ossidi, polimorfi, solvatati o sali farmaceuticamente accettabili di tali composti.

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Milano, 1 agosto 1997

RECORDATI, Industria Chimica e Farmaceutica S.p.A.